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CHARLES ALBERT SHULL

AN APPRECIATION

The quotation opposite Professor SHULL's portrait was the FOREWORD in the first number of PLANT PHYSIOLOGY. It enunciated the goal and ideal which he envisaged for the new journal as he embarked upon its editorship. Under his able editorial guidance, these objectives have been faithfully maintained by him for nearly two decades and have now become firmly established as the editorial policy of the journal. Professor SHULL as editor strove consistently to improve the professional quality of the published papers and to diversify the scope of the journal by publication of impartial reviews of important books, by inclusion of illustrated biographies of outstanding plant physiologists, and by addition of news notes on important botanical events. His efforts have been dedicated to broadening the scope of the subject and to the development of new concepts and techniques which serve to unify physiology with the physical sciences as well as with other aspects of botany itself. The journal now serves as an extremely useful and authoritative outlet for plant physiological workers throughout the world. The high place achieved by PLANT PHYSIOLOGY among scientific journals is an eloquent tribute to Professor SHULL's foresight and industry.

At the beginning of this century, the development of plant physiology as a pure and applied science indicated that it was destined to become more than a minor subdivision of botany, and that considerable progress was also to be achieved by greater application of physical and chemical methods. In succeeding years, it also became increasingly apparent that the progress of physiology could be greatly accelerated by the creation of an organized body of plant physiologists who recognized the importance of maintaining a strong unity both between the instructional and research phases of the subject itself and their applications in agriculture and industry. The establishment of a new outlet for publications was necessitated by the increase in volume and the change in the type of the research in the field of plant physiology, much of which was of interest to physicists and chemists as well as to botanists.

With the enthusiastic support of many professional workers, the AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS was formally organized in 1924 with Dr. CHARLES A. SHULL as its first president. His initiative and resolute endeavor soon led to its firm establishment and eventual enlargement into a world-wide organization unique in its service to the profession. In 1926, the difficulties of launching a new journal without outside subsidy were surmounted and the society's official journal, PLANT PHYSIOLOGY, was also estab-

lished. With about four hundred pages in its initial volume, a journal of dignified format and high professional standards was made possible through the generous support of Professor and Mrs. SHULL who also anonymously absorbed the recurring deficits of the early issues. In ensuing years the journal enjoyed a continuous growth and wide acceptance throughout the world under Professor SHULL's resourceful editorship. Under his leadership, also, membership in the society increased rapidly, the size of the volumes of PLANT PHYSIOLOGY was soon doubled and the list of institutional subscribers was greatly enlarged.

Though Dr. SHULL was ably assisted in the editorial preparation of manuscripts by an equally devoted group of associated editors, he continued the arduous task of proof-reading manuscripts, supervising the printing, and for many years personally assuming the responsibility of the necessary financial guarantees. Through his grasp of financial matters and with the assistance of numerous small contributions from within the profession, Professor SHULL early inaugurated several specific endowment funds to provide permanent income for the Society, its journal, the Hales Prize awards, the honorary Barnes Life Memberships and publication of a separate series of PLANT PHYSIOLOGY monographs. Although growth of these endowments has been slow, it has been steady and they now assure continuation of the various projects of the society. Professor SHULL's repeated personal donations have encouraged many others to contribute various sums in support of the society's programs.

Despite his editorial work, Professor SHULL continued his own exemplary research contributions and his proctorship of many promising young plant physiologists. Through his farsighted and tireless devotion to the AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS and to its journal, Dr. SHULL has given a strong and lasting impetus to the growth of plant physiology on a world-wide scale. Few scholars have given as generously of their time, energy and substance in the interest of their profession and their colleagues as Professor SHULL. Though his responsibilities must often have proved burdensome and exhausting, he, nevertheless, continued to meet them with a high degree of self-imposed loyalty and effectiveness, all without financial recompense.

It is gratifying to note that Professor SHULL's outstanding services as an editor and scientist have not passed unnoticed by American plant physiologists. In addition to perpetuating his term as Editor-in-Chief until Dr. SHULL himself requested release upon the occasion of retirement from his professorship, American plant physiologists have twice elected him president of the society, bestowed upon him an honorary Barnes Life Membership, and awarded him the Stephen Hales Prize for distinction in research. Upon the occasion of his retirement as Editor-in-Chief of PLANT PHYSIOLOGY after two decades of distinguished service and as a further expression of appreciation, the AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, in harmony with formal action taken at the Cleveland meeting of the society, gratefully dedicates Volume Twenty of PLANT PHYSIOLOGY for 1945 to Professor CHARLES ALBERT SHULL.

ANALYSIS OF THE ORGANIC ACIDS OF ORANGE JUICE[1]

WALTON B. SINCLAIR, E. T. BARTHOLOMEW, AND R. C. RAMSEY

(WITH FOUR FIGURES)

The total acidity of orange juice is ordinarily determined by titrating a known volume of juice with a standard solution of NaOH, with phenolphthalein as indicator, the result being expressed as citric acid. This value includes all the substances of an acidic nature in the juice that react with NaOH. It so happens, however, that in most fruits the chief substances reacting with NaOH are the organic acids; for this reason, the titratable acidity represents fairly well the organic acid content of a given juice.

The predominating organic acid in orange juice is citric acid; but other organic acids, namely, tartaric, malic, benzoic, and succinic, have been reported present (**2**). According to SCURTI and DE PLATO (**11**), the acidity of the orange is due to citric and malic acids. MENCHIKOVSKY and POPPER (**6**) have reported that in the grapefruit of Palestine, 98.72 per cent. of the total acid is citric, 0.05 per cent. tartaric, 1.0 per cent. malic, and 0.23 per cent. oxalic. NELSON (**7**) found that the nonvolatile fruit acids of California lemons were chiefly citric, with a trace of l-malic acid. For Florida orange juice, HARTMANN and HILLIG (**5**) reported a citric acid concentration of 0.92 per cent. and a malic acid concentration of 0.18 per cent., fresh weight. HALL and WILSON (**3**) identified formic acid in the volatile fraction of orange juice.

All of these acids, if present in the juice, would have an additive influence on the titratable acidity. It was therefore decided to determine the amounts of citric and malic acids in orange juice and correlate these with the free-acid content as determined by titration with standard NaOH. Undoubtedly, other organic acids are present in the juice in minute amounts, but in the present work it has been assumed that they are negligible in comparison with the concentrations of citric and malic acids. Furthermore, the concentrations of combined and free acids have been determined on various juice samples, and the relation between the combined acid and the inorganic cations in the ash of orange juice has been ascertained.

Materials and methods

For each experiment, aliquot portions of centrifuged juice were taken from the volume of juice reamed from 50 mature Valencia or navel oranges. Total soluble solids were determined with an Abbé refractometer, the refractive indexes being converted to soluble solids by means of a sucrose table. The free-acid content of the juice samples was determined by three different methods: (1) by titration, with phenolphthalein as indicator; (2)

[1] Paper no. 518, University of California Citrus Experiment Station, Riverside, California.

by potentiometric titration; and, (3) by the lead acetate precipitation method of HARTMANN and HILLIG (**4**).

In the potentiometric titration, the acidity was calculated from the inflection point of the titration curve. For each sample of juice, the inflection point was determined for the titration curve by plotting the ratio $\frac{\Delta \text{pH}}{\Delta \text{ml.}}$ against the milliliters of NaOH added. All pH values were determined at 23° C., with a Beckman glass-electrode pH meter. In the method of HARTMANN and HILLIG (**4**), the addition of lead acetate to the juice precipitates the neutral lead salts of the fruit acids, with the formation of an equivalent quantity of acetic acid. The salts of the fruit acids are also precipitated by lead acetate, but this is apparently a replacement reaction, for no acetic acid is liberated in the process. The liberated acetic acid was determined in the deleaded filtrate by titrating an aliquot portion with standard NaOH solution. The results were calculated and reported as citric acid.

The actual amount of citric acid and citrates in the juice samples was determined by the method of PUCHER, VICKERY, and LEAVENWORTH (**8**). The sample was heated with H_2SO_4 to convert the combined citrates to free citric acid, and the citric acid was oxidized to pentabromacetone by $KMnO_4$ in the presence of KBr. After extraction of the pentabromacetone with petroleum ether, the bromide ion was liberated with Na_2S, and subsequently titrated with standard $AgNO_3$. The citric acid in the original sample was calculated from the titration.

The malic acid was precipitated from the juice by a procedure described by HARTMANN and HILLIG (**5**). The organic acid fraction was precipitated from an alcoholic solution (75 per cent.) with lead acetate, and the precipitate separated from the filtrate by centrifuging. The precipitate was washed with alcohol, suspended in water, and freed of lead by passing H_2S through the solution. The lead sulphide was filtered off and washed with water. The filtrate and washings were combined and diluted to a known volume. The malic acid was determined on aliquot portions of this solution by the method of PUCHER, VICKERY, and WAKEMAN (**9**). The principle of the method involved the oxidation of malic acid with $KMnO_4$, in the presence of KBr, to a bromine compound volatile with steam. This compound reacts with dinitrophenylhydrazine to give a water-insoluble product which is soluble in pyridine. A pyridine solution of this substance, when correctly diluted with water and made alkaline with NaOH, develops a blue color proportional to the amount of malic acid present.

The combined-acid concentration of various juice samples was estimated by determining the alkalinity of the ash from an aliquot of juice. A 50-ml. aliquot of juice was evaporated to dryness on a steam bath, and then charred over a low flame before ashing to a white residue at 450° C. An aliquot of standard HCl was added to the ash, and the excess acid was titrated with NaOH. The alkalinity was calculated from the equivalents of HCl neutralized by the ash. This value represents closely the equivalents of combined acid present in the original sample of juice.

Results

Relation of free acidity of orange juice to the acid constituents precipitated with lead acetate

The organic acids and phosphoric acid were precipitated as the neutral lead salts when lead acetate was added to the juice. The free-acid values (as citric) were calculated from the amounts of standard NaOH solution required to neutralize the liberated acetic acid. According to Hartmann and Hillig (**4**), the organic acids and acid salts are precipitated by lead acetate, and free acetic acid is thus liberated. When this liberated acetic acid is determined by titration, the original free-acid content of the juice can be calculated. These values were determined on each sample of juice reported in table I.

The differences in the amounts of acid determined in the juice by direct titration and by the lead acetate method are statistically insignificant. This means that lead acetate precipitates the same acid substances that are neutralized when the juice is titrated directly with standard NaOH to phenolphthalein end point. If the juice contains high acid phosphates, these would be included in the values obtained by titrating the liberated acetic acid in the filtrate from the lead acetate precipitate, whereas, in titrating the juice directly with NaOH, only part of the phosphoric acid would be included. The phosphates occur with the organic acids in fruits as the acid phosphates, and these substances contribute to the titratable acidity. A correction should therefore be made for the acid phosphate in the juice, depending upon the amount present. The second hydrogen of phosphoric acid is slightly undertitrated at the end point of phenolphthalein (pH 8.10). When titrating potassium dihydrogen phosphate electrometrically, Bollinger (**1**) observed that 47.4 per cent. of the total hydrogen ions was neutralized at pH 8.10. When titrating this same material with phenolphthalein, Hartmann and Hillig (**4**) obtained results accounting for 50 per cent. of the total available hydrogen ions. The results of these experiments show that the acid substances determined by the lead acetate method are a true measure of the free acidity of orange juice.

Since the phenolphthalein titration for orange juice is always high, owing to the fact that the indicator gives a color change above the inflection point of the titration curve, it would appear to be difficult to compare the free acidity (as citric acid) obtained by direct titration and by the lead acetate method. However, when the various factors are studied carefully, it is evident that there is considerable basis for comparison. Probably the most important of these factors is the influence of small amounts of acid phosphates in orange-juice samples.

When orange juice is titrated potentiometrically, all free acids and approximately 40 to 45 per cent. of the acid phosphate salts are neutralized at the inflection point of the juice. The difference in acidity between the lead acetate value and the potentiometric value must therefore be due to acid salts, such as phosphates, that are not completely neutralized at the inflection

TABLE I

COMPARISON OF TOTAL FREE ACID, AS DETERMINED BY DIFFERENT METHODS, AND CITRIC ACID CONTENT OF ORANGE JUICE

SAMPLE NO. AND TYPE	SOLUBLE SOLIDS	SPECIFIC GRAVITY	pH	TOTAL FREE ACID (AS CITRIC ACID) DETERMINED BY						CITRIC ACID (PENTABROMACETONE METHOD)	
				PHENOLPHTHALEIN TITRATION		LEAD ACETATE METHOD		POTENTIOMETRIC TITRATION			
	%			*mg./ml.*	%*	*mg./ml.*	%*	*mg./ml.*	%*	*mg./ml.*	%*
1. Valencia	12.58	1.0508	3.50	11.8	1.12	11.8	1.12	11.0	1.05	12.0	1.14
2. Valencia	12.58	1.0508	3.55	12.4	1.18	12.5	1.19	11.7	1.11	13.1	1.25
3. Navel	16.77	1.0687	3.96	8.3	0.78	8.3	0.78	7.6	0.71	8.9	0.83
4. Navel	16.32	1.0667	3.97	7.7	0.72	7.8	0.73	7.2	0.68	8.9	0.83
5. Valencia	11.91	1.0480	3.40	11.3	1.08	11.3	1.08	10.8	1.03	11.8	1.13
6. Valencia	15.44	1.0630	3.41	13.8	1.30	13.9	1.31	13.3	1.25	14.7	1.38
7. Valencia	12.31	1.0496	3.23	12.3	1.17	12.4	1.18	12.1	1.16	12.7	1.21
8. Valencia	12.51	1.0505	3.32	12.1	1.15	12.1	1.15	11.8	1.12	12.0	1.13
9. Valencia	14.32	1.0581	3.45	12.7	1.20	12.7	1.20	12.1	1.14	13.4	1.27
10. Valencia	13.92	1.0564	3.52	12.8	1.21	12.8	1.21	12.2	1.15	13.5	1.28
11. Navel	15.72	1.0641	3.99	8.5	0.80	8.4	0.79	7.5	0.70	9.8	0.92
12. Valencia	13.07	1.0528	3.40	11.4	1.07	11.6	1.09	11.2	1.06	12.4	1.18
13. Valencia	13.18	1.0533	3.45	11.4	1.08	11.3	1.07	10.9	1.04	12.7	1.21
14. Valencia	12.51	1.0505	3.80	9.4	0.89	9.5	0.90	8.9	0.85	9.6	0.91

* Percentage fresh weight.

points on the titration curves but are precipitated by lead acetate. Some of this difference may be due to other factors, such as slight overtitration of the acetic acid; but it is apparent that in a substance such as orange juice, which contains a small amount of phosphate and other acid salts, the electrometric titration does not account for all of the hydrogen ions. HARTMANN and HILLIG (4) arrived at this same conclusion in their work with jams and jellies and other fruit products.

From the data in table I, it can be seen that the values determined by phenolphthalein titration and by the lead acetate method agree very closely, even though the direct titration value is considered higher than the actual acidity. Evidently, the phenolphthalein titrates just high enough above the actual inflection point of the juice to account for the untitrated acid phosphates. In principle, it amounts to one error compensating for another.

A number of experiments were carried out on the precipitate formed by the addition of lead acetate to the juice. This precipitate included the lead salts of the fruit acids, combined acids, and acid salts of the juice. Quantitative data on these precipitated constituents are given elsewhere in this paper. The precipitate was freed of lead by passing H_2S through the material suspended in water. The insoluble PbS was filtered off, leaving the free acids in the filtrate. The citrates and acid salts which previously existed as such in the natural juice were then in the form of free acids and could be titrated in the absence of buffer salts. The titration curve of these constituents gave a curve similar to that of pure citric acid. This was what would be expected, for the titration of free acids in an unbuffered system, such as that of citric acid, gives a steeper curve near the inflection point than in a highly buffered system such as that of orange juice.

THE FREE ACID OF ORANGE JUICE DETERMINED BY PHENOLPHTHALEIN TITRATION AND BY POTENTIOMETRIC TITRATION

The free acid (as citric) per milliliter of juice, calculated from the volume of standard NaOH required for neutralization, with phenolphthalein as indicator, was higher than the amounts determined by potentiometric titration (tables I, II). Since phenolphthalein changes color between pH 8 and 9, slightly more NaOH is required to reach the end point with phenolphthalein than to titrate the corresponding samples potentiometrically to pH 7.80. Immediately after the juice samples had been titrated with NaOH, with phenolphthalein as indicator, the pH values were determined and found to range between pH 8.25 and pH 8.45 (table II). It is essential that the sample be sufficiently diluted with distilled water to prevent the natural coloring matter in the juice from interfering with the end point produced with phenolphthalein. If this precaution is not observed, it is very easy to overtitrate the solution to a pH value ranging from 8.8 to 9.2.

The value for the mean pH and the standard deviation for the inflection points on the titration curves of orange juice was 7.80 ± 0.03. This value agrees with that of ROBERTS (10), who showed that the inflection points on

TABLE II

COMPARISON OF THE PHENOLPHTHALEIN AND POTENTIOMETRIC TITRATION METHODS OF DETERMINING THE FREE ACIDITY (AS CITRIC) OF ORANGE JUICE

SAMPLE NO. AND TYPE	PHENOLPHTHALEIN TITRATION		POTENTIOMETRIC TITRATION		CITRIC ACID CALCULATED*
	CITRIC ACID	pH AT END POINT	CITRIC ACID	pH AT INFLECTION POINT	
	mg./ml.		*mg./ml.*		*mg.*
1. Navel	8.6	8.28	8.2	7.82	8.5
2. Navel	10.0	8.45	9.4	7.79	9.9
3. Valencia	10.8	8.40	10.4	7.80	10.7
4. Valencia	10.3	8.25	9.9	7.82	10.1
5. Valencia	9.7	8.44	9.2	7.84	9.5
6. Navel	9.1	8.36	8.6	7.79	9.0
7. Valencia	11.7	8.41	11.2	7.80	11.6
8. Valencia	11.1	8.43	10.6	7.77	10.9

* Calculated by taking NaOH value from potentiometric curve at point of pH of phenolphthalein end point.

the titration curves of various fruit juices occurred at approximately pH 7.90.

The free acidity was calculated from the amounts of standard NaOH required to bring each sample of juice to the inflection point. Since more alkali was required to bring the juice to the phenolphthalein end point on

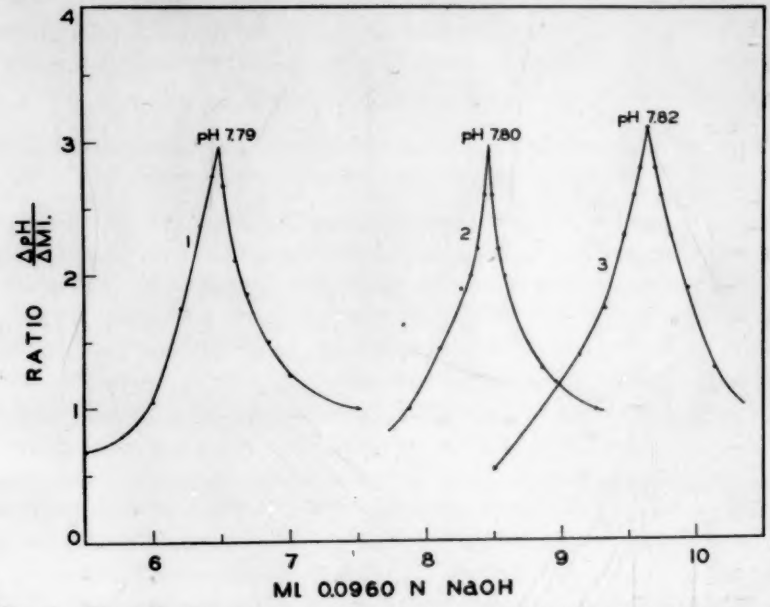

FIG. 1. The inflection points on the titration curves of three typical samples of Valencia-orange juice. The points were determined by plotting the change in ratio $\frac{\Delta \text{ pH}}{\Delta \text{ ml.}}$ against the milliliters of NaOH added.

the curve (a point between pH 8.25 and 8.45), it is to be expected that the titrations using phenolphthalein as an indicator would yield higher free-acid values (table II). Experimentally, the more accurate values for the amounts of free acid in the juice samples would be given by values calculated from the amounts of NaOH required to bring the samples to the inflection points. These are the values used in determining the combined acids of orange juice.

The inflection points on the titration curves of three typical samples of Valencia-orange juice are shown in figure 1. The values shown on each graph were from the portion of the titration curve near the neutral point

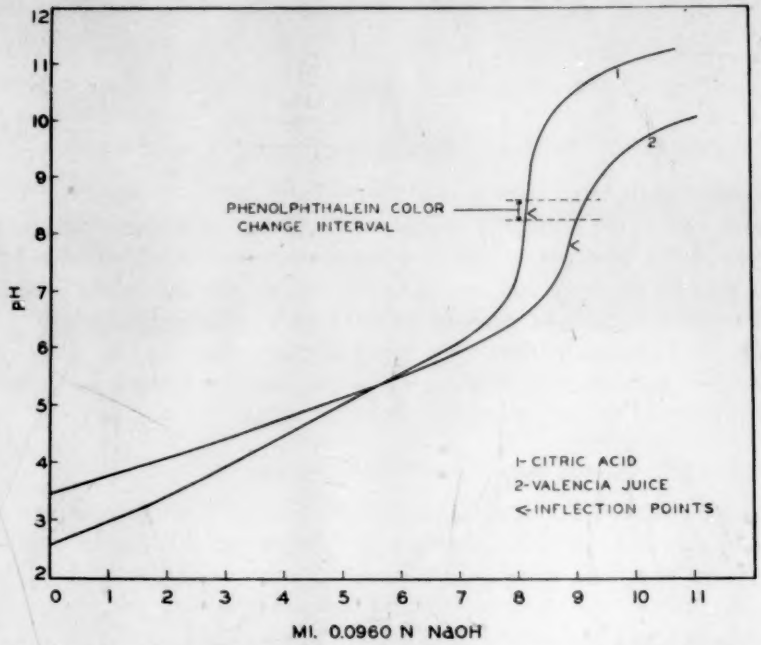

FIG. 2. Comparison of the buffer properties (1) of pure citric acid; and (2) of natural Valencia-orange juice. Note the difference in the inflection points of the two curves and the relation of the inflection points to the phenolphthalein end point.

and were determined by plotting the ratio $\frac{\Delta \text{ pH}}{\Delta \text{ ml.}}$ against the milliliters of standard NaOH added. This ratio represents the increment of pH for a given addition of NaOH solution. That each of the three samples had different quantities of free acid was shown by the different amounts of NaOH required to bring them to their inflection points (see curves 1, 2, and 3, fig. 1). It is of interest to note the insignificant differences between the pH values of the juice samples at their inflection points.

The acidity of fruits is chiefly due to the polybasic acids which constitute the major portion of the total fruit acids. This is especially true of orange juice, in which total acidity is due mostly to citric acid. As previously noted, the electrometric titration curve of orange juice shows a sharp in-

flection close to pH 7.80. This is the only sharp inflection in the titration curve of orange juice, for the three dissociation exponents (pKa values) of citric acid are sufficiently close to cause the removal of the multiple inflections on the curve. In general, the titration curves of extracts of most acid fruits show a sharp inflection close to pH 8.0. According to the experiments of BOLLINGER (1), the total acidities of juice and water extracts of fruits, calculated from electrometric end points of pH 8.10, agreed closely with those determined by the phenolphthalein titration.

The titration curve of pure citric acid (fig. 2) is nearly perpendicular to the abscissa in that portion of the curve close to the neutral point. The titration curve of orange juice shows considerable slope within a similar region of the curve. It can be seen that the titration curve of pure citric acid would produce a much larger ratio $\frac{\Delta \text{ pH}}{\Delta \text{ ml.}}$ at the inflection point than would orange juice. In all the samples studied, the value of the ratios $\frac{\Delta \text{ pH}}{\Delta \text{ ml.}}$ at the inflection points varied almost 100 per cent. between samples. Unlike the orange-juice titration, the phenolphthalein indicator gives an accurate end point when pure citric acid is titrated with NaOH. This is to be expected because all buffering agents of any importance are absent, and the titration curve is almost perpendicular near the inflection point. Since the titration curve of orange juice is not so steep near the inflection point, it is evident that more NaOH would be required to bring such a highly buffered solution to the phenolphthalein end point.

THE CITRIC ACID CONTENT OF ORANGE JUICE

The amounts of citric acid (milligrams per milliliter) actually determined in the different juice samples are shown in table I. It should be pointed out that the citric acid values, as determined by the method of PUCHER, VICKERY, and LEAVENWORTH (8), include both the free citric acid and that combined in the form of citrates. These values are higher than the quantity of free acid calculated as citric acid from the amount of standard NaOH required to bring the sample to pH 7.80 on the electrometric titration curve; they are also slightly higher than the amounts determined from the titratable acidity. The data reported for the actual amount of citric acid in the juice represent the major portion of the total organic acid content of orange juice.

The degree of accuracy with which the citric acid and the citrate content of orange juice can be determined by the pentabromacetone method is reported in table III. The data given include the determination of citric acid on a solution of known strength, and on aliquot portions of this solution, to which increments of 1 to 5 ml. of sodium citrate solution had been added. The concentration of the sodium citrate solution was prepared so that 1 ml. of the solution was equivalent to 1 mg. of citric acid. Similar determinations were made on samples of orange juice to which increments of sodium

citrate had been added. It can be seen that the amount of citrate added, both to the standard solutions and to the orange-juice samples, could be quantitatively determined by the pentabromacetone method. The values obtained by this method therefore include both the free citric acid and the combined citrates. It follows that the difference between the actual citric acid value and an accurate measure of the free acid represents the citrate content of a given juice sample only when all the free acid in the juice is citric acid.

THE MALIC ACID CONTENT OF ORANGE JUICE

The malic acid content of various orange-juice samples is recorded in table IV. The citric acid values are reported for the same samples; and for comparison, these values were obtained on the pure juice and on the acid

TABLE III

RECOVERY OF CITRIC ACID AND ADDED SODIUM CITRATE FROM DIFFERENT SAMPLES OF ORANGE JUICE

SAMPLE	MILLIGRAMS CITRIC ACID PER MILLILITER, DETERMINED BY						
	PHENOLPHTHALEIN TITRATION	LEAD ACETATE PRECIPITATION	POTENTIOMETRIC TITRATION	PENTABROMACETONE METHOD, ON SAMPLES HAVING GIVEN AMOUNTS OF SODIUM CITRATE ADDED*			
				None	1 ml.	3 ml.	5 ml.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Standard citric acid	10.0	10.0	10.0	10.0	11.1	13.1	15.0
Valencia-orange juice	12.7	12.7	12.1	13.4	14.5	16.5	18.4
Valencia-orange juice	12.8	12.8	12.2	13.5	14.4	16.6	18.6
Navel-orange juice	8.5	8.4	7.5	9.8	10.7	12.9	14.9

* Sodium citrate was a concentration equivalent to 1 mg. citric acid per milliliter. The increments of sodium citrate solution were added to 1 ml. of juice.

constituents precipitated with lead acetate. The degree of maturity of the fruit from which the samples were taken can be partly indicated by the soluble-solids content, pH of the juice, and the acidity. The slight variation in the malic acid content of the different samples, in contrast to the large differences occurring in corresponding amounts of citric acid, is noteworthy. For example, the malic acid values for samples 3, 4, and 5 were 1.58, 1.40, and 1.58 mg. per milliliter, respectively, whereas the citric acid values for these samples were 25.51, 9.27, and 12.99 mg. per milliliter. As the amounts of malic acid in the juice samples are fairly constant, the differences occurring in free acidity (potentiometric titration) are due chiefly to differences in their citric acid contents.

Attention should be drawn to the close agreement between the citric acid values determined on pure juice and those determined on substances precipitated with lead acetate. Apparently, lead acetate gave a complete precip-

TABLE IV

COMPARISON OF CITRIC ACID AND MALIC ACID CONTENT OF ORANGE JUICE

SAMPLE NO. AND TYPE	PURE JUICE						ACID CONSTITUENTS PRECIPITATED BY LEAD ACETATE				COMBINED ACID (AS CITRIC)		FREE ACID PERCENTAGE OF TOTAL ACID RADICALS*
	SOLUBLE SOLIDS	pH	CITRIC ACID DETERMINED BY				CITRIC ACID (PENTABROM-ACETONE METHOD)		MALIC ACID				
			POTENTIOMETRIC TITRATION		PENTABROM-ACETONE METHOD								
	%		*mg./ml.*	*m.e.*† */ml.*	*mg./ml.*	*m.e./ml.*	*mg./ml.*	*m.e./ml.*	*mg./ml.*	*m.e./ml.*	*mg./ml.*	*m.e./ml.*	%
1. Navel‡	11.23	3.07	12.28	0.192	13.83	0.216	13.59	0.212	1.03‡	0.015	2.50	0.039	83.1
2. Valencia	15.38	3.70	7.98	0.126	9.53	0.149	9.51	0.148	1.50	0.022	2.88	0.045	73.7
3. Valencia	10.57	2.88	24.18	0.378	25.39	0.397	25.51	0.398	1.58	0.024	2.75	0.043	89.8
4. Valencia	14.78	3.78	7.80	0.122	9.42	0.147	9.27	0.145	1.40	0.021	2.94	0.046	72.7
5. Navel	13.24	3.33	11.45	0.179	12.97	0.203	12.99	0.203	1.58	0.024	3.07	0.048	78.8
6. Navel	12.84	3.23	13.03	0.204	14.82	0.231	14.84	0.232	1.77	0.026	3.39	0.053	79.4
7. Valencia	14.97	3.84	6.87	0.107	8.38	0.131	8.28	0.129	1.42	0.021	2.88	0.045	70.4
8. Navel	12.17	3.28	11.17	0.174	12.42	0.194	12.46	0.195	1.77	0.026	2.94	0.046	79.3
9. Valencia	10.83	2.91	23.38	0.365	24.72	0.386	24.60	0.384	1.62	0.024	2.88	0.045	89.1

* The percentage of free acid is calculated from the ratio $\frac{\text{free acid} \times 100}{\text{total acids}}$, in which free acid indicates milliequivalents of citric acid calculated from the potentiometric titration of the juice. The total acid value is the sum of the milliequivalents of total citric acid (pentabromacetone method) and total malic acid.

† m.e. = milliequivalents.

‡ With the addition of 1 mg. of malic acid per milliliter of juice, malic acid recovery for this sample was 1.99 mg. per ml.

itation of the citric acid radical (free and combined) in the juice. Malic acid determinations are recorded only for the lead acetate precipitate, for in the pure juice there were substances which yielded values on the blank determinations greatly in excess of the actual amount of malic acid in the samples. That lead acetate precipitated all the malic acid was demonstrated by adding known amounts of the acid to juice samples and subsequently determining the malic acid in the lead acetate precipitate of these samples, as shown in table IV, sample 1. There is reason to believe that these malic acid values represent to a fair degree of accuracy (within 5 per cent.) the actual amount of malic acid in the juice samples.

Free and combined organic acids of orange juice

The relative amounts of free acid in the different juice samples are recorded in table IV. The percentage of free acid is calculated from the ratio $\frac{\text{free acid} \times 100}{\text{total acids}}$, in which free acid indicates milliequivalents of citric acid calculated from the potentiometric titration of the juice. The total acid value is the sum of the milliequivalents of total citric acid (determined by the pentabromacetone method) and total malic acid. The concentration of the individual organic acids in the free-acid fraction is unknown; the free acid is therefore calculated in terms of citric acid. The error in this calculation is insignificant, since the malic acid content is low in comparison with the citric acid content, and the equivalent weights of the two acids are nearly equal (citric acid, 64.02; malic acid, 67.02). Total citric acid and total malic acid are calculated as the pure acids, even though part of each exists as the acid salts. When the absolute amounts of these acid salts are given, the values represent the weights of pure citric or malic acid that are combined with the cation, and not the absolute weight of the salt itself.

The relation of pH value to the percentage of free acid in different samples of orange juice is shown graphically in figure 3, curve A. The pH range of this curve shows that the juice samples were taken from orange fruits that ranged from green to overmature. Each point on this curve represents a different juice sample. Curve B shows the percentage of free acid existing at different pH values on a titration curve of pure citric acid (0.2 N). With each increment of NaOH added to the citric acid, the concentration of free acid decreased, while that of the citrate salt increased. The apparent difference between these two curves can be partly explained by the inorganic salts occurring naturally in the juice. The combined effect of these other constituents probably causes a variation in pH away from that of a pure solution of citric acid and its salt.

Within the pH range in which orange fruits are generally accepted as being mature (pH 3.2 to 3.8), the relation (fig. 3, A) is a straight-line function. Both above and below this pH range, the line is more or less curved. The curve on the upper part of the line is probably due to the fact that fruits giving such a high pH value are very near the decomposition point.

In fact, the highest point on that curve represents a sample of fruit in which breaking down of the pulp had already started, and probably is not representative. The reason for the change in slope of curve A at about pH 3.2 has not been experimentally determined. However, if the straight portion of the curve were extended downward with the same slope, it would eventually cross the curve for pure citric acid and citrate.

The pH of even very green fruits never approached that of pure citric acid of the same concentration. With a large number of juice samples there would probably be some variation on either side of the curve. This could be caused by variations in juice constituents, other than the organic acids

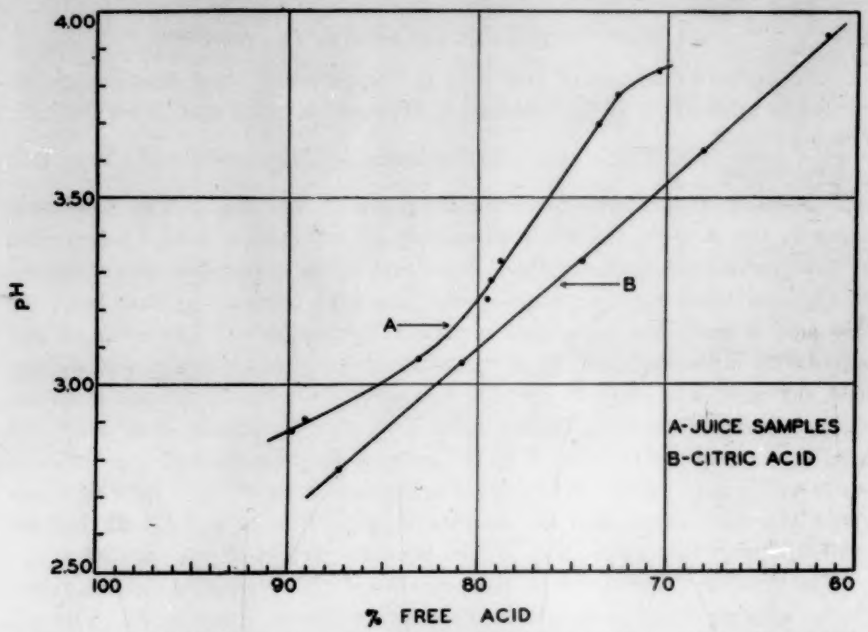

FIG. 3. The relation between pH and the percentage of free acid in orange juice (A), compared with similar data determined on citric acid solution (B). The percentage of free acid is the percentage of the total acid radical that exists as free organic acid. Each dot on curve A represents one of the juice samples listed in table IV, for which the pH, free-acid concentration, and total acid concentration were determined.

and their salts, that affect the pH, and also by the experimental error involved in determining the amounts of free and combined acid in a juice. For example, a small amount of acid phosphate is titrated when the free acidity of a juice is determined potentiometrically. If a correction were made for this phosphate, the free acidity value for the organic acids would be lowered slightly. This would cause curve A to fall closer to curve B. No correction for the acid phosphate was made in this work, because this constituent varies in samples from different districts and even from different trees.

The magnitude of the ratio of combined acid to free acid, at different

pH values of orange juice and of pure citric acid, is shown in figure 4. The initial points on curves B and C designate the values of this ratio at the pH of natural juice before titration. The other points on the curves were obtained by potentiometric titration of the juice sample with standard NaOH. For each increment of alkali, the amount of free acid, in milliequivalents, that was changed to sodium citrate was easily calculated. The combined acid for all points, other than the initial ones, included the combined acid present in the original sample and the sodium citrate formed by the neutralization of part of the free acid. The maximum free-acid concentration

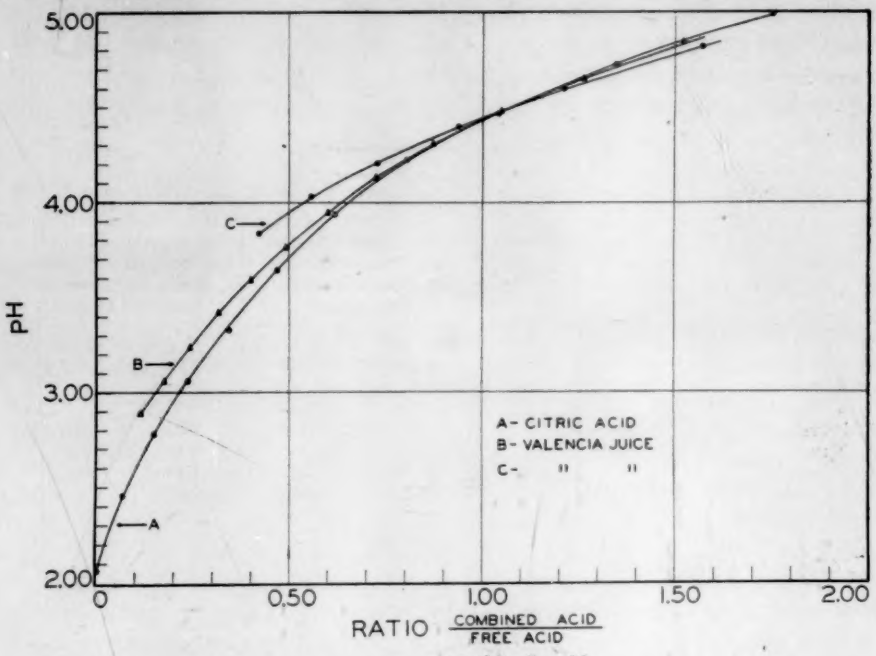

FIG. 4. The relation of pH to the ratio $\frac{\text{combined acid}}{\text{free acid}}$, for two juice samples, compared with a similar curve for pure citric acid solution. The first dot for curves B and C represents the values determined on each juice sample before the addition of any NaOH. For the other points, the term "combined acid" includes the amount occurring in the original sample and the sodium citrate formed by the addition of standard NaOH in titrating the sample potentiometrically.

of the sample is given by the titer value of NaOH required to reach the inflection point. In these studies, the free acid was calculated as milliequivalents of citric acid, and the combined acid as the milliequivalents of citric acid in salt form. As indicated by the initial pH of the juice, samples B and C were from immature and overmature fruit, respectively. Curve B agreed more closely than curve C with the curve for pure citric acid (A). This is undoubtedly due to the fact that the juice from green fruit contained nearly three times as much acid as that from overmature fruit. As the acid content decreased, the acid salts and the other constituents in the

juice had an increasing effect upon the pH, even though their concentration remained nearly constant. It is of interest that the three curves converge at a point where the ratio is almost equal to 1. In other words, when NaOH has been added so that the milliequivalents of free acid and combined acid are equal, the solutions have nearly the same pH. This pH value (4.40 to 4.50) is in close agreement with the dissociation exponent of the second dissociation constant for citric acid ($pKa_2 = 4.39$).

If the free- and total-acid values are known, it is a matter of simple calculation to determine, indirectly, the amount of organic acids in the combined form. Experimentally, the problem involves the determination of the individual organic acids and other acid radicals which compose the total acidity (free- and combined-acid radicals). The total organic acid content of the juice was taken as the sum of the milliequivalents of total citric acid

TABLE V

COMBINED-ACID CONTENT OF ORANGE JUICE, DETERMINED FROM ALKALINITY OF ASH

SAMPLE NO. AND TYPE	SOLUBLE SOLIDS	pH	TOTAL FREE ACID (AS CITRIC) DETERMINED BY POTENTIOMETRIC TITRATION		COMBINED ACID (AS CITRIC) CALCULATED FROM CARBONATE CONTENT OF ASH	
	%		*mg./ml.*	*m.e.*/ml.*	*mg./ml.*	*m.e./ml.*
1. Valencia	10.57	2.88	24.18	0.378	2.77	0.043
2. Valencia	14.97	3.84	6.87	0.107	2.87	0.045
3. Valencia	10.83	2.91	23.38	0.365	2.70	0.042
4. Navel	11.31	3.36	10.43	0.163	2.71	0.042
5. Navel	12.78	3.45	9.62	0.150	2.66	0.042
6. Valencia	14.38	3.63	8.44	0.132	2.48	0.039
7. Valencia	12.37	3.03	19.02	0.297	2.92	0.046
8. Valencia	13.07	3.07	16.45	0.257	2.89	0.045

* m.e. = milliequivalents.

and total malic acid determined on each sample. There are undoubtedly minute quantities of other organic acids present, but the concentration of these acids is so small as to be negligible. Therefore, the calculations in table IV were based on the following formula (in milliequivalents):

Total acids = (free citric acid + free malic acid) + (combined citric acid + combined malic acid)

With this formula, the amount of combined acid was determined by difference.

The data in table IV show that the amount of combined acid in the different juices was remarkably uniform, the juice from overripe fruit containing no more than the juice from green fruit. Apparently, the free acids are not converted to combined acids, for the absolute amount of free acids actually decreased as the fruit matured. This is further substantiated by calculating the combined acids from the alkalinity of the ash of the juice (table V). The alkalinity of the ash is a measure of the inorganic bases combined with the organic acid radicals, which decompose to carbonate on ashing. The carbonate in the ash is equivalent to a mean of 0.043 milli-

equivalents of acid (2.76 mg. citric acid) in the combined form, per milliliter of juice. The combined acids determined by these two methods (tables IV and V) therefore agreed satisfactorily.

It is apparent that the amount of organic acids that combine with the mineral bases to form salts depends upon the concentration of available cations. The total cation content of the juice is derived from the ash constituents, but not all of these constituents are combined with the organic acids. Some are combined with anions such as the phosphates, sulphates, and a small amount of chlorides. As shown in table I, the citric acid calculated from the titration value, with phenolphthalein as indicator, was significantly lower than the total amount of citric acid and citrates in the juice. Greater differences occurred between the actual amount of citric acid and the amount determined from the electrometric titration curve (at pH 7.80 ± 0.03) of the various samples. It is obvious, therefore, that the amount of free organic acids available for salt formation in the juice would never be a limiting factor, for the acid concentration at all times would be many times greater than the total cation content.

Summary

The total free acids in the juice of mature Valencia and navel oranges were calculated (1) from the titratable acidity, with phenolphthalein as indicator; (2) from the amount of NaOH required to bring a known volume of orange juice to the equivalence point (pH 7.80 ± 0.03), when titrating potentiometrically; and (3) from the amount of NaOH required to neutralize the liberated acetic acid formed when the organic acids and acid salts in the juice are precipitated by lead acetate. The values determined by these three methods are compared with the actual amount of citric acid and combined citrates chemically determined in the various samples.

The acid constituents of orange juice, as determined by titrating the juice directly with standard NaOH to phenolphthalein end point, were equivalent to the amounts determined by the lead acetate method. The total free acids determined from the points of inflection on the titration curves were significantly lower than those reported both for the phenolphthalein titration and for the lead acetate method. When juice samples were titrated, with phenolphthalein as indicator, the end points ranged from pH 8.25 to 8.45. The potentiometric values have been taken to represent more accurately the total acidity.

The concentration of malic acid in different juice samples varied only slightly, as compared with the changes in the citric acid content. With one exception, the samples studied had a malic acid content of from 1.40 to 1.77 mg. per milliliter of juice, while the citric acid varied from 8.38 to 25.39 mg. per milliliter. It is evident that variations in acidity of the orange are due chiefly to changes in the citric acid concentration.

Apparently, there is a definite relation between the free acid-combined acid balance and the pH of the juice. The amount of combined acids in the

juice is remarkably uniform; this indicates that the free-acid concentration is the chief variable. With a decrease in the value of $\frac{\text{total free acid}}{\text{total free and combined acids}}$, a corresponding rise in pH occurs.

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EFFECT OF FERTILIZERS ON LOBLOLLY PINE IN A NORTH CAROLINA NURSERY

RUSSELL ROSENDAHL AND CLARENCE F. KORSTIAN

Introduction

The proper application of commercial fertilizers to forest nursery soils is of considerable importance since it may profoundly influence the value of the seedlings produced.

According to LUNT (2): "Experiments in soil management in forest nurseries go back at least 65 years. VON SCHROEDER (1893) cites the work of DULK on Scotch pine, Norway spruce, and beech in 1874 and 1875, and of SCHUSSE on pine and spruce in 1872, 1879, and 1882." Since this time a large amount of information has been accumulated concerning the response of tree seedlings to various fertilizers in many localities and on a wide variety of soils. This information is of much local value but permits of few generalizations.

TOUMEY and KORSTIAN (8) note the lack of information concerning the quantitative requirements of various species and age classes of nursery stock for essential elements and conclude: ". . . Until such information is available, fertilizer experiments must remain on an empirical basis and should be conducted for each nursery and each set of conditions. . . ." Within the past few years some attempts have been made to determine quantitatively the nutrient requirements of certain species of seedlings (2, 3, 4, 5, 6, 7, 9, 10). MITCHELL (3, 4) used pot culture tests and chemical analyses of seedlings to determine optimum concentrations of various elements and the availability of soil nutrients. NÉMEC and other investigators determined available nutrients by various soil extraction methods and correlated these values with growth. LUNT (2), in addition, found the actual amounts of certain elements removed by seedlings and transplants per acre per year. WILDE (9, 10) has outlined soil-fertility standards for northern conifers and hardwoods, values being obtained by determining available nutrients in a number of productive forest soils of the Lake States.

Fertilizer requirements may be determined: (1) by field tests to find the fertilizer treatment which results in optimum growth; (2) by chemical analyses of seedlings to determine concentrations of various elements; and (3) by chemical analyses of nursery soils for available nutrients. The first method is time-consuming; it is accurate, however, and the only one feasible in many cases. Also, it is fundamental to the second and third methods which require calibration to determine values corresponding to good seedling growth. Each soil extraction method and each species and age class of stock requires separate calibration. The general applicability of results is problematical. Chemical analyses of seedlings eliminate the "availability" question but other difficulties are encountered, such as the effect on

concentration of one element by one or more limiting elements. Considerable care is necessary in the interpretation and application of results.

The present field test was made to determine the effect of three commercial fertilizers—ammonium sulphate (N), superphosphate (P), and potassium chloride (K)—on the growth of loblolly pine in the nursery of the United States Soil Conservation Service located near Chapel Hill, North Carolina. These three fertilizers, each applied in uniform amounts throughout the experiment, were tested singly and in all possible combinations forming a typical factorial design (1). Furthermore, each experimental plot was split; one half received a treatment a month earlier than the other—the total amount of fertilizer applied being identical for each half-plot. Further experiments, not reported here, were made to determine the optimum rate of application of several commercial fertilizers. The effects of the fertilizers on several qualities other than dry weight were also studied.

Methods

The experimental design required eight whole plots, one for each fertilizer or combination of fertilizers as mentioned above; the experiment was replicated six times. Each plot was 4.5 by 16 feet, or 72 square feet in area, and contained 48 linear feet of drill. Whole-plots (treatments) within a replication were completely randomized, as was the position of each half of a whole-plot. Fertilizers were applied dry and thoroughly worked into the soil two weeks prior to seeding in the following amounts in pounds per acre: ammonium sulphate (N), 550; superphosphate (20 per cent. P), 600; and potassium chloride (K), 600.

Combination treatments (NP, NK, PK, and NPK) received the same amount of each component as single treatments. Plots were seeded in early March and thereafter received ordinary nursery care. Seedlings were lifted, graded, and samples randomly drawn from plantable stock in November at the end of one growing season. Each seedling was measured for taproot length, shoot length, average length of two needles from the upper part of the stem, diameter of stem at root collar, mycorrhizal abundance (on an arbitrary scale), and dry weights of roots and shoots. In addition, the percentage of stock classified as plantable was recorded. Measurements other than dry weight were made to determine the relationship between the several qualities measured under varied fertilizer treatment.

Results

Results of the treatments are given in table I. The nitrogen effect was significantly positive for every item measured; the potassium effect was significantly negative for 5 out of 7 items.

The phosphorus-potassium effect was significantly positive for one out of 7 items; that is, it was less negative than would be the case if the phosphorus and potassium effects were additive.

Mean values for seedlings receiving ammonium sulphate alone were invariably significantly greater than means for control plots, while mean

values for seedlings receiving ammonium sulphate in combination treatments were not significantly greater than control means.

Potassium chloride had the reverse effect; mean values for seedlings receiving this salt alone were smaller than control plot values for all 7 items. Seedlings treated with potassium chloride and superphosphate together did not differ significantly from seedlings from untreated beds for any items, except stem diameter.

Values for seedlings receiving superphosphate alone were mostly less than those for control seedlings, although they were not statistically significant.

TABLE I

EFFECT OF FERTILIZER TREATMENTS ON 1–0 LOBLOLLY PINE SEEDLINGS

TREATMENT	DEVIATIONS FROM MEANS FOR UNFERTILIZED STOCK						
	ROOT WEIGHT	SHOOT WEIGHT	TOTAL DRY WEIGHT	DIAMETER OF STEM AT ROOT COLLAR	SHOOT LENGTH	NEEDLE LENGTH	PLANTABLE SEEDLINGS
	gm.	*gm.*	*gm.*	*cm.*	*cm.*	*cm.*	%
N	+0.273*	+0.812*	+1.12*	+0.061*	+1.765*	+0.76*	+7.733*
P	−0.011†	−0.136†	−0.12†	−0.004†	−0.163†	+0.32†	−0.458†
K	−0.075§	−0.278‡	−0.38‡	−0.037‡	−0.676†	−0.24†	−6.044‡
NP	−0.020†	−0.013†	+0.01†	−0.008†	−0.109†	−0.03†	+0.610†
NK	−0.051†	−0.058†	−0.15†	−0.010†	+0.210†	+0.04†	−0.121†
PK	+0.049†	+0.170†	+0.18†	+0.021**	+0.401†	+0.31†	+2.417†
NPK	−0.050†	−0.111†	−0.20†	−0.010†	+0.365†	−0.12†	+1.227†
Standard error	0.030	0.096	0.128	0.009	0.475	0.225	2.093

* Statistically very significant increase (1 per cent. level).
** Statistically significant increase (5 per cent. level).
† Statistically insignificant.
‡ Statistically very significant decrease (1 per cent. level).
§ Statistically significant decrease (5 per cent. level).

Root-shoot ratios ranged from 0.30 (control plot mean) to 0.36 (phosphorus-potassium plot mean).

Mycorrhizal abundance varied considerably within a plot and showed little or no correlation with treatment.

Good correlation was found between total dry weight of seedlings, yield, root-collar diameter, and plantable percentage, on the basis of statistically significant effects and plot means. Only slight correlation was found between yield and shoot length or needle length. The effects of fertilizer treatments on root length and abundance of mycorrhizae were not treated statistically; tap roots were broken at a rather uniform depth and the measurement of mycorrhizal abundance appeared too crude for analysis.

Discussion

The Chapel Hill Nursery is located on Congaree silt loam, a fertile alluvial soil of the Piedmont plateau; hence, specific results of this experiment are not widely applicable. It is evident that, of the fertilizers tested,

ammonium sulphate is the only one worth using. Potassium chloride and superphosphate alone or in combination, in the amounts used, are either worthless or financially unjustified. Apparently sufficient amounts of potassium and phosphorus are now available to meet the requirements of loblolly pine without the application of commercial fertilizers. The duration of this fertility under intensive cropping is, of course, problematical.

The fact that there were no significant differences in yield between plots receiving the same total amount of fertilizer in one or two applications is probably due to the relatively high base-exchange capacity of Congaree silt loam and the immobility of the fixed phosphates. Apparently only slight leaching took place. It should be emphasized that entirely different results might be obtained on the sandy nursery soils more common in the northern United States.

The relationship between seedling dry weight, diameter, and percentage of plantable seedlings is obvious. An effective fertilizer would be expected to result in large, heavy plants, and a high percentage of plantable stock. The close correlation found here indicates that a fairly reliable measure of fertilizer efficiency (on the basis of dry weight) can be obtained by finding the percentage of seedlings graded as plantable.

Summary

The determination of fertilizer requirements of loblolly pine in the Chapel Hill Nursery by means of fertilizer field tests has been outlined.

Ammonium sulphate alone, in the amount applied, was shown to be the only effective or financially justified treatment of the 7 single and combination treatments used. When applied in combination with superphosphate, potassium chloride, or both, the seedlings were not significantly larger than control-plot seedlings. Apparently the addition of the last two fertilizers did not have a positive effect.

Potassium chloride alone, in the amount applied, had a depressing effect on growth. When added with superphosphate, this effect was lessened. Superphosphate alone, in the amount added, had little effect on seedling growth.

The addition of the same total amount of fertilizers in two applications instead of a single application had no statistically significant effect on growth.

Seedling diameter and plantable percentage were closely correlated with dry weight, while needle length and shoot length showed only slight correlation with dry weight.

The assistance of PROFESSOR F. X. SCHUMACHER in designing the experiment is gratefully acknowledged. The nursery work was under the supervision of LLOYD R. ROOF, Manager of the Chapel Hill Nursery of the U. S. Soil Conservation Service.

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THE INFLUENCE OF HEPARIN ON GROWTH OF *LUPINUS ALBUS* SEEDLINGS

DAVID I. MACHT

(WITH TWO FIGURES)

At first engaging the attention primarily of zoöphysiologists and hematologists (**6, 7, 14**), the study of heparin has lately also been taken up by biochemists who have largely succeeded in determining its complicated chemical structure. More recently, this interesting drug has acquired some practical significance as a prophylactic agent against thrombo-embolic complications of various kinds, such as pulmonary embolism, thromboses of the retinal vessels, and thrombo-embolic puerperal complications (**8, 13**). These discoveries instigated the author's more extensive pharmacological study of heparin, a study which has already yielded information of practical value (**9**). Thus the writer found that the toxicity of digitalis, ouabain, and other digitaloid drugs is definitely lowered by prior heparinization of the animals treated with those drugs. This phenomenon was explained by the discovery that all of the digitaloid preparations tended to shorten coagulation time of the blood of animals studied, a finding which recently GILBERT, DE TAKATS and their associates have more definitely corroborated (**4, 16**). The present writer has also shown that injections of purified heparin prevent or modify anaphylactic shock in guinea pigs (**10**).

In connection with a research on the general physiology and pharmacology of heparin, the author, who has been studying for years the comparative zoöpharmacological and phytopharmacological effects of drugs and chemicals (**11, 12**), has been investigating extensively the growth of Lupinus seedlings in various concentrations of heparin, the results of which study are described in this communication.

Chemistry of heparin

Although not yet completely solved, the chemistry of heparin is fairly well known (**1, 2, 3, 5**). Heparin is a mucoitin polysulphuric acid with not less than 40 per cent. sulphuric acid. Being a polysaccharide, it possesses an extremely high negative charge, which is probably responsible for its reaction with other compounds playing a rôle in the coagulating system. In fact, heparin may be regarded as a sort of hormone regulating the blood coagulation and, like the hormones, it is produced by a special kind of cell; namely, the so-called mast cells of the connective tissue, found mainly in the vicinity of capillaries and blood vessel walls. Heparin is thought to pass from these cells either directly or by diffusion into the blood stream. The so-called metachromatic granules of these cells, consisting of heparin, exhibit characteristic staining reactions.

In the present investigation the substance was obtained from various sources but, in general, there was no quantitative difference in the pharma-

cological effects of American, Canadian, Swedish, Swiss, and other makes of heparin. The majority of experiments performed were made with heparin prepared by the laboratories of Hynson, Westcott & Dunning, Inc. Their heparin is assayed by HOWELL'S original method in which freshly drawn cat's blood is kept fluid by one milligram of the substance for 24 hours under standardized conditions. In most of the author's experiments the heparin employed had a potency of 1:30 and 1:50.

Methods

Large, medium and small varieties of *Lupinus albus* seeds were used in various experiments although most of the tests were made with the medium variety. The results yielded by the different sizes were practically the same. The seeds were soaked in tap water for 24 hours after which they were planted in finely ground sphagnum moss containing 75–80 per cent. of moisture. After germinating and developing roots from 30 to 50 mm. long, the seedlings were ready for testing. Control seedlings were placed in upright hard glass test tubes containing a solution made up of equal parts of Shive's solution (**15**) and distilled water. Under exactly the same ecological conditions, other seedlings from the same crop were grown in similar solutions containing, in addition, various percentages of heparin which is freely soluble in water. After the length of each of the roots of the various seedlings in control and heparin solutions had been carefully measured, all the seedlings in the numerous rows of the outfit were allowed to grow in the dark at a temperature of 20° C. for 24 hours. The roots of the seedlings were then measured again with a millimeter rule and the increment of root elongation in the respective solutions was noted and compared. The ratio of growth of seedlings in a solution of any given drug divided by the average growth of control seedlings is designated as the phytopharmacological or phytotoxic index. Numerous tests were made in this manner, in every case at least ten seedlings being grown in each solution. Experiments were repeated until the average obtained became statistically reliable. The results reported in this paper represent the averages derived from the 10 to 15 tests made for each solution of heparin used.

TABLE I

EFFECT OF CONCENTRATIONS OF 1:20,000 OF HEPARIN ON ELONGATION OF *Lupinus albus* SEEDLING ROOTS AT 20° C. FOR 24 HOURS

BRAND OF HEPARIN	INDEX OF GROWTH
	%
H. W. & D., Lot C (1:100)	108
H. W. & D., Lot D (1: 80)	110
H. W. & D., Lot E (1:100)	109
H. W. & D., Lot X (1: 50)	117
Swedish	108
Canadian	112
Swiss	108
Sodium polyanethol sulphuric acid	66

TABLE II

GROWTH OF *Lupinus albus* SEEDLINGS IN SOLUTIONS OF HEPARIN

CONCENTRATION	INDEX OF GROWTH	CONCENTRATION	INDEX OF GROWTH
	%		%
1: 100,000	112	1: 45,000	111
1: 95,000	112	1: 40,000	103
1: 90,000	108	1: 35,000	82
1: 85,000	110	1: 30,000	85
1: 80,000	108	1: 25,000	110
1: 75,000	82	1: 20,000	113
1: 70,000	80	1: 15,000	83
1: 65,000	75	1: 10,000	80
1: 60,000	77	1: 5,000	76
1: 55,000	93	1: 3,500	74
1: 50,000	101	1: 1,000	66

Results

A comparison of the growth of various series of *Lupinus albus* seedlings in solutions of the different lots of heparin revealed qualitatively similar tendencies as may be seen from table I, which shows Lupinus root elongation in concentrations of 1:20,000. The index of root growth in all the solutions was higher than that of the controls although the several lots differed somewhat quantitatively. A comparison of seeding growth in concentration of true heparin with that of plants in a solution of the synthetic substance, sodium polyanethol sulphuric acid, which bears some resemblance to heparin in chemical structure and anticoagulant effect, revealed its greater toxicity for root growth. It was demonstrated in all of the experiments that there was very little difference in hydrogen ion concentration of the various solutions and that the pH certainly played no rôle in their toxicity.

A more detailed study was made on growth of *Lupinus albus* seedlings in solutions of heparin (H. W. & D.). The effects of concentrations ranging from 1:1,000 to 1:100,000 were repeatedly tested and the mean indices or averages of results of all experiments made are given in table II. These results are plotted in the subjoined graph in which the ordinates represent

TABLE III

GROWTH OF *Lupinus albus* IN SODIUM POLYANETHOL SULPHURIC ACID

CONCENTRATION	INDEX OF GROWTH
	%
1: 10,000	61
1: 20,000	63
1: 30,000	64
1: 40,000	65
1: 50,000	71
1: 60,000	72
1: 70,000	75
1: 80,000	76
1: 90,000	78
1: 100,000	83

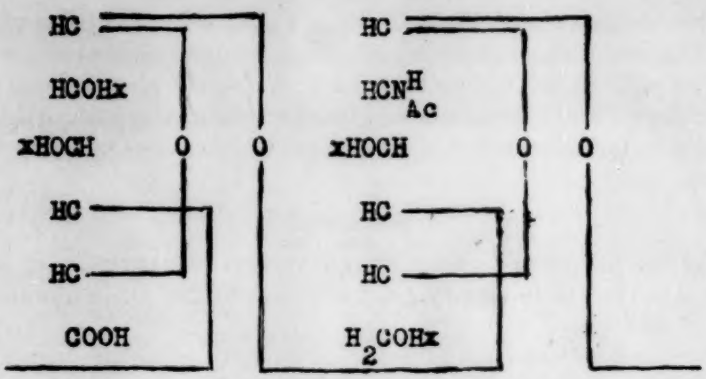

FIG. 1. The organic moiety of heparin, mucoitin = glucuronic acid + acetylated glucosamine.

the index of growth and the abscissae the various concentrations of heparin studied. The curve is of interest because of its peculiar peaks and depressions. Thus there is a definite stimulation of root growth between concentrations of 1:100,000 and 1:80,000. A second peak of growth stimulation rises between concentrations of 1:50,000 and 1:40,000 and still a third between 1:25.000 and 1:20,000. On the other hand, the definite depression or inhibition of growth found in the region between concentrations of 1:75,000 and 1:60,000 indicates their toxicity for root growth of *Lupinus albus* as compared with that of the controls. A similar depression occurs between concentrations of 1:35,000 and 1:30,000. A toxicity is also exerted

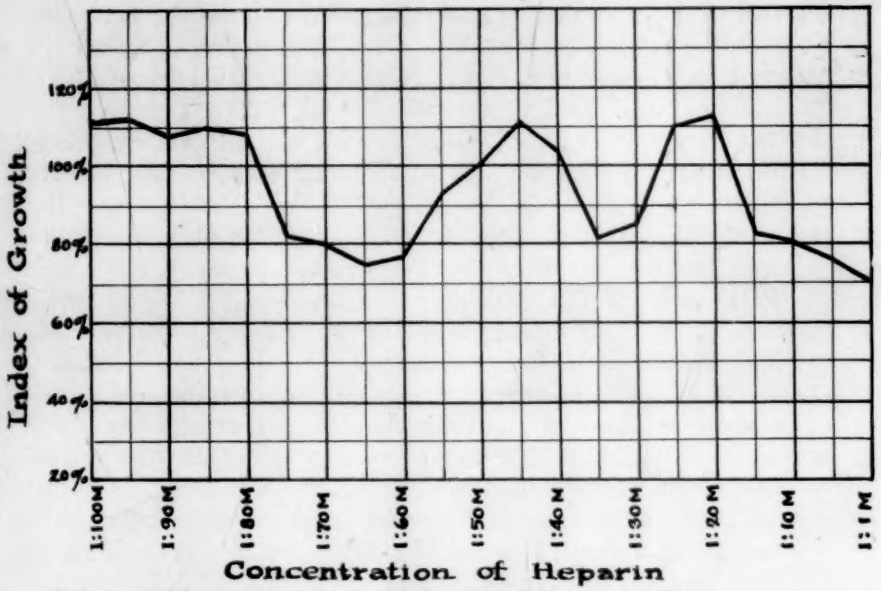

FIG. 2. Influence of heparin on growth of *Lupinus albus* seedlings.

by greater concentrations than 1:20,000. The significance of this variation in the phytopharmacological effects of heparin solutions is not clear. Further studies on this subject will be necessary. Table III gives the phytotoxic indices obtained with various concentrations of sodium polyanethol sulphuric acid and shows no stimulation of growth, even of seedlings in highly diluted solutions.

Summary

1. The root growth of *Lupinus albus* in various concentrations of purified heparin was studied in standardized solutions at 20° C. in the dark for periods of 24 hours.

2. Certain concentrations of heparin definitely stimulate growth while others inhibit it, thus yielding several peaks and depressions in the curve of growth when it is plotted.

3. Such an effect was produced by all the specimens examined.

4. The sodium salt of polyanethol sulphuric acid, a chemical homologue of heparin, however, was found to be more toxic for plants than heparin itself and did not stimulate growth but inhibited it progressively in proportion to the increase in concentration of the solutions used.

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SIGNIFICANCE OF THE pH OF BLUEBERRY LEAVES[1]

AMIHUD KRAMER AND A. L. SCHRADER

(WITH TWO FIGURES)

Ecologically, the swamp, or highbush blueberry, *Vaccinium corymbosum* L., differs fundamentally from other cultivated plants in its requirements for an acid soil, a constant source of moisture (**6**), and a relatively high anion nutrient uptake (**19**). In conjunction with studies on blueberry nutrition presented elsewhere (**19**), data were obtained on the effect of mineral nutrient deficiencies and age of leaves on the pH of the blueberry leaf sap. It was thought that these studies on the pH of the leaf sap might offer the key to an understanding of the mechanism by which the swamp blueberry can live in its usual environment.

Materials and methods

Dormant rooted blueberry cuttings of the Cabot variety were planted on January 7, 1942, in 54 coffee urn liners (glazed crocks) containing quartz sand, and accommodating two cuttings per crock. Beginning ten days later, nine different nutrient solutions were applied, designed to induce the following mineral nutrient deficiencies: None, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, and boron. The composition of the solutions and the method of application have been previously described (**19**). As an added precaution, each solution was adjusted to a pH of 4.8–5.0 with hydrochloric acid. In all, six liners (12 plants) were treated with each of the nine solutions. Four plants from each treatment were removed for analyses on February 22 (46 days after planting), four others on March 15 (67 days after planting), and the remaining four on April 10, 1942 (93 days after planting).

Determinations of the pH of the plant sap were made by means of a Coleman pH electrometer (model 3). Immediately after the removal of each plant, the sap for pH determinations was prepared by grinding a representative part of the current leaf growth in a mortar with a few milliliters of distilled water and quartz sand and decanting the supernatant liquid into the cuvette of the pH apparatus.

The titration curves (fig. 1) were constructed from data obtained as follows: Two grams of fresh leaves of the respective plant species growing in the greenhouse were thoroughly macerated with 100 ml. of distilled water in a Waring blendor, transferred, and made up to 250 ml. with additional distilled water. A 50-ml. aliquot was removed, to which increasing quantities of N/15 hydrochloric acid were added, with subsequent measurement of the pH following each addition of the acid. Another 50-ml. aliquot was similarly treated with increasing additions of N/15 sodium hydroxide.

[1] Scientific Paper no. A80, Contribution no. 1937 of the Maryland Agricultural Experiment Station (Department of Horticulture).

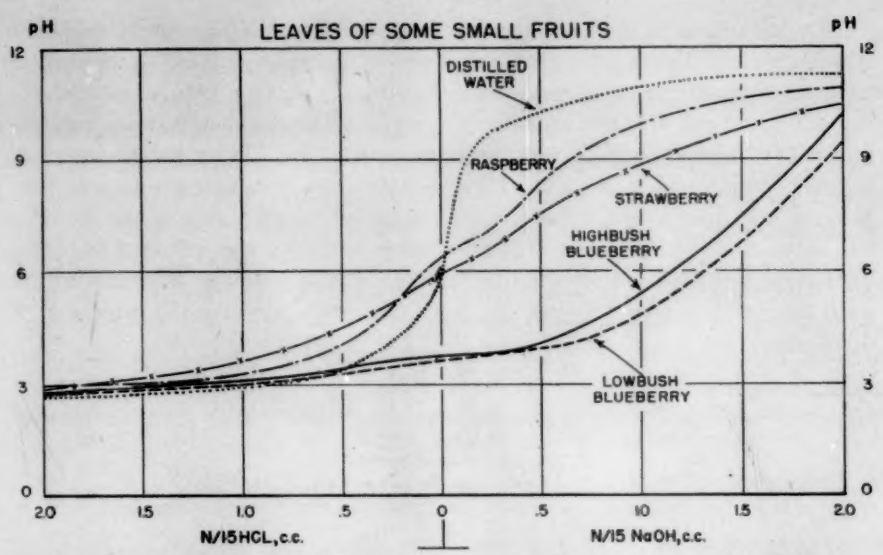

FIG. 1. Titration curves of blueberry leaves compared to other small fruits.

In order to separate the water-soluble constituents of the blueberry leaf tissue from the residual material (fig. 2), additional blueberry leaves were macerated, filtered, and made up to 250 ml. The residual material was dispersed for several minutes in additional 250 ml. of distilled water. Only 25-ml. aliquots were used for these titrations and consequently the titration curves thus obtained are not directly comparable to those presented in figure 1.

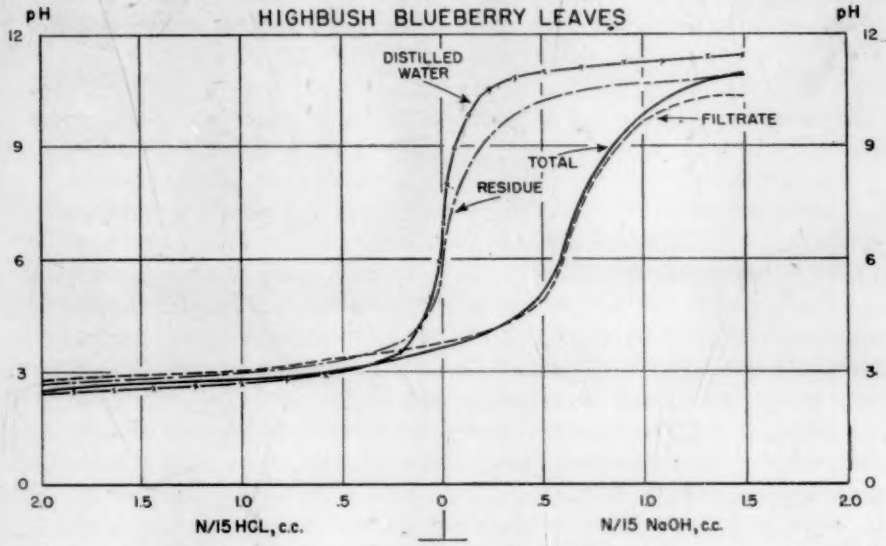

FIG. 2. Titration curves of the water soluble filtrate compared to the residual material of blueberry leaves.

An indication of the isoelectric point of the water-soluble proteins in the blueberry leaf tissue was obtained from a filtrate prepared in a similar manner from 20 grams of fresh leaves and made up to a volume of 500 ml., by adding 25-ml. aliquots to each of a series of beakers containing varying amounts of hydrochloric acid or sodium hydroxide. After the aliquot was added to each beaker the pH of its contents was determined; it was then filtered, the precipitate dried down to constant weight, and weighed. The pH value at which the greatest precipitation occurred was assumed to be the isoelectric point for water-soluble proteins. It is believed that the severe agitation of blueberry leaves during maceration caused the proteins to denature.

TABLE I

EFFECT OF AGE AND MINERAL NUTRIENT DEFICIENCIES ON THE pH OF BLUEBERRY LEAVES. FIGURES REPRESENT AVERAGES OF FOUR ROOTED CUTTINGS PLANTED IN SAND CULTURES ON JANUARY 7, 1942

DEFICIENCY	REMOVED ON FEB. 22, 1942		REMOVED ON MARCH 15, 1942			REMOVED ON APRIL 10, 1942	
	LEAVES	LEACHINGS	LEAVES		LEACHINGS	LEAVES	
			YOUNG	OLD		YOUNG	OLD
None	3.66	4.4	3.45	3.70	4.01	3.43	3.77
Nitrogen	3.69	6.0	3.56	3.67	5.93	3.44	3.67
Phosphorus	3.70	4.4	3.55	3.74	3.98	3.77	4.45
Potassium	3.67	4.2	3.75	3.96	4.02	3.59	4.31
Calcium	3.90	4.5	3.81	4.26	4.25	3.87	4.06
Magnesium	3.78	4.4	3.80	4.17	4.31	3.65	4.07
Sulphur	3.67	4.5	3.70	3.86	4.08	4.00	4.40
Boron	3.90	4.6	3.50	3.85	4.13	3.53	3.87
Iron	3.65	4.2	3.65	3.82	3.99	3.50	3.75
F value	8.37*	.....	1.25	3.90*	1.46*	2.85*	3.37*
Significant difference	0.15	.....	.....	0.30	0.27	0.27	0.37

* Statistically significant at odds of 99: 1.

Results

The younger blueberry leaves have a lower pH than the more mature leaves (table I). The difference in the pH of young and old leaves was least in the nitrogen-deficient plants.

When young leaves were sampled separately, no significant effect of mineral deficiencies on pH was found until the last removal (93 days after planting) when the young leaves deficient in phosphorus, calcium, or sulphur were higher in pH than the young full nutrient leaves. This may have been an indication that those plants ceased active growth, and thus even the leaves towards the tip of the shoot were older than they would have been had growth continued normally.

When old leaves were sampled separately, calcium- or magnesium-deficient leaves were significantly higher in pH by the second removal (67 days after planting) and with the exception of the minus-nitrogen and minus-iron

plants, all of the older nutrient deficient leaves showed a higher pH than the old full nutrient leaves by April 10 (93 days after planting). In general, the leaf areas that were necrotic for any reason whatever were least acid, the pH of such areas reaching as high as 5.0.

The leachings from all of the pot cultures, with one exception, approximated the pH value of 4.4 at the first removal and 4.0 at the second removal. The pH leachings of the minus-nitrogen cultures, however, averaged close to pH 6.0.

The pH titration curves for leaf sap of blueberries and other small fruits are given in figures 1 and 2. It is obvious that not only is the hydrogen-ion concentration of blueberry leaf sap strikingly greater than that of the other plant species, but the titratable acidity of the blueberry is also much higher. The data presented in figure 2 indicate that practically all the acidifying substances are readily water soluble.

When aliquots of the water-soluble filtrate of blueberry leaf tissue were added to solutions of different pH's, the greatest precipitation occurred at pH 5.0, thus indicating that the isoelectric point of the water-soluble proteins in blueberry leaves is at approximately that point.

Discussion

The above results are significant when viewed in the light of the concepts developed by ROBERTSON (**21**), PEARSALL (**20**), and others, regarding the relation of the hydrogen-ion concentration of plant sap to the isoelectric point of the plant proteins. Briefly, they found that a double maximum of physico-chemical activity (*e.g.*, imbibition, swelling, etc.) exists when the plant sap pH is at either side of the pH of the isoelectric point of the plant proteins. This plant activity diminishes as the pH of the plant sap approaches the pH of the isoelectric point of the plant proteins. Actually, however, saps of all plants examined by them and later by others (**7, 9, 18**), had a decidedly higher pH than that of the isoelectric point of the respective plant proteins.

For the blueberry, however, the reverse seems to be true; in fact, the pH of the blueberry plant sap is two to three full points below that of most cultivated plants, as illustrated in figure 1. In other words, the hydrogen-ion concentration in blueberry leaves is 100 to 1000 times greater than in leaves of most cultivated plants. Whereas in many plants the younger and more vigorous parts are higher in pH than older plant parts (**7, 12, 17, 18, 21, 24**), in the blueberry the younger parts are lower. Conversely, whereas various deficiencies (**8, 9, 10, 11, 18, 22**) and incidences of disease (**13, 14, 16, 21, 23, 24**) caused a decrease in the pH of other plants, such conditions usually resulted in an increase in the pH of the blueberry plant sap. It appears, therefore, that in contrast to most plants, the pH of blueberry leaf sap *rises* towards the pH of the isoelectric point of its proteins upon senescence or the occurrence of disease or injury, since the isoelectric point of the soluble blueberry leaf proteins was found to be approximately at the pH of 5.0, while the pH of young, healthy leaves was about pH 3.5.

Since the leaf sap of the blueberry does appear to be well on the acid side of the isoelectric point of its soluble proteins, are not the following speculations justified? Is it not probable that the amphoteric proteins, in contrast to their behavior in most plants (**3, 20**), act in the blueberry as cations? Then if some of the plant proteins act as cations, would that not partially explain the preferential absorption of anions (**19**); and if anion radicals are absorbed in excess, would it not be difficult to keep iron in the blueberry plant in the reduced, available form, thus explaining the frequent reports of iron deficiency in blueberries (**2**)? Furthermore, in the presence of relatively large quantities of anion radicals which in themselves may serve as oxidizing agents (**1, 15**), would there not be less need for the oxidizing effect of the manganese ion, thus explaining the absence of a beneficial effect on blueberry growth from the application of manganese (**19**)?

From the practical standpoint it is interesting that the pH value of the minus-nitrogen plants remained low, and there was little difference in pH between the younger and older minus-nitrogen leaves; pH might therefore serve as a diagnostic tool for confirming suspected nitrogen deficiency in blueberries.

In an attempt to combine into one basic concept some of the results of blueberry plants when compared with results obtained with other plants, especially in the light of some recent findings on the mechanism of mineral ion intake and the significance of the hydrogen ion concentration of plants, the following generalization was postulated: The behavior of the swamp blueberry may be based upon its capacity to thrive under soil conditions limiting aeration, but supplying at the same time adequate moisture to meet rather exacting water requirements of the plant. Experiments supporting this view indicate that the blueberry has exacting moisture and oxygen requirements, high anion and low cation uptake, a requirement for highly acid soils, an extremely acid plant sap, and that the pH of its plant sap is on the acid side of the isoelectric point of its soluble proteins.

Considering the natural occurrence of the blueberry on soils with high water tables, how can the experimental evidence be used in explanation of a special adaptation of the blueberry to such conditions? Because of the high moisture requirement blueberries usually thrive on soils with high water tables. Lack of aeration in such soils is overcome not by the presence of aerenchyma tissue, but by the extremely shallow root system and a high soil acidity where oxidation rather than reduction processes prevail (**5**). Since such soils are low in calcium and other exchangeable bases, a low cation requirement is necessary for plants to exist in such an environment, while the satisfaction of the high anion requirement may increase the usually limited supply of oxygen needed for respiration and organic acid formation. The high organic acid content is responsible for the low pH value of the plant sap, which is below the isoelectric point of the "soluble" plant proteins. These proteins may consequently act as cations, thus balancing the presence of excessive anions. The prevailing shade of the natural environ-

ment aids in further maintaining the low pH by preventing organic acid destruction (**4**).

When the blueberry is transplanted from its natural habitat into ordinary cultivated soils, it probably suffers from lack of moisture, and since it may ordinarily obtain a part of the oxygen used in respiratory processes from anion radicals, suffers from lack of such nutrients. The destructive effect of high light intensities on the organic acid content of the plant and the consequent increase in pH towards the isoelectric point may also be a factor of measurable effect on the growth of blueberries in cultivated fields where no shade is provided.

In conclusion, it seems reasonable to raise the following question: From a physiological standpoint, is the blueberry (and perhaps similar plants, the cranberry for example) radically different from most cultivated plants, and if it is different, should not comprehensive physiological studies of such plants be undertaken in order to arrive at a broader understanding of plant processes in general?

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SOME ASPECTS OF THE METABOLISM OF THE EBENEZER ONION[1]

EMMETT BENNETT

(WITH TEN FIGURES)

The onion is an old world species which has been cultivated for over 4000 years. It is said to have been worshipped in Egypt prior to the Christian era and even to have been used in the Druid rites. Today the onion is recognized as the chief food plant in which the food is stored in a bulb (**5**).

The first work on the metabolism of the onion appears to be that of ZALESKI (**12**), in which he showed that a considerable synthesis of protein took place in the sprouting of the bulbs in the dark and during the rest periods. He believed that amino acids were used in this synthesis since the content of amides remained unchanged. These findings were confirmed by PRIANISCHNIKOW, SCHRODER, and FRIEDRICH (**12**). Later ZALESKI and SHATKIN (**13**) in a similar experiment again obtained evidence to support the former conclusion. The possibility of amides or ammonia playing a rôle in the synthesis seemed to be precluded by the data of these authors because variations were within the limit of experimental error. In 1932 RAHN (**9**) concluded that as in seed germination, amino nitrogen was translocated to the leaves where it was used in protein synthesis.

This investigation was designed to provide information on the general chemical composition of the Ebenezer onion and to determine some of the chemical changes which occur during normal growth and also under certain abnormal conditions.

Experimental methods

The onion sets were grown in Merrimac fine sandy loam soil. The first sample was taken when the sets weighed about 25 gm. and subsequent samples at intervals of about four to eight days depending upon the rate of development until the completion of growth. The number in a sample depended upon size and the degree of maturity. The first samples consisted of 175; the last prior to pulling 98, and after harvest, 62 onions. Samples were taken at about 9:00 A.M., on each occasion and on days of similar weather. They were brought to the laboratory immediately, where the adhering soil was wiped off and, in the case of the first samples, the old scales were removed. Fresh weights of leaves, bulbs, and roots were determined. Moisture determinations were made by the BIDWELL and STERLING method (**1**), using 25-ml. receiving tubes. Each portion was sliced, then dried by hot air in a forced-draft oven at about 60° C. The dried samples were then ground in a Wiley mill, and stored in bottles.

In the case of the culture work, the onions were taken when the weight of each plant was about 100 gm., and before the tops had fallen over. A

[1] Contribution no. 517 of the Massachusetts Agricultural Experiment Station.

sample of 60 onions was prepared in the usual way in order to serve as a control. In addition, eight samples of 40 each were cleaned, weighed, and placed in large buckets containing enough distilled water to partially cover the bulbs. Four of these were placed in a greenhouse. Conditions there approximated those under which the plants had been grown. The other four samples were placed in a basement without light of any kind. This room had a temperature of 18° C., and a relative humidity of 85 per cent. At intervals of about 24 hours entire samples were removed and treated as described in the preceding paragraph.

Methods of analyses

Total nitrogen was determined by the Kjeldahl method modified to include nitrate nitrogen (**1**). For water-soluble nitrogen tests the dry tissue

TABLE I

DATA RELATIVE TO SAMPLING, CULTURAL CONDITIONS, GROWTH OF ONIONS AND THEIR CONDITION THROUGHOUT THE PERIOD OF GROWTH

AGE	DATE, 1943	TEMPERATURE		MOISTURE		PERCENTAGE OF PLANT		WEIGHT PER PLANT	CONDITION OF TOPS
		AIR	SOIL	TOP	BULB	TOP	BULB		
days		*°C.*	*°C.*	*%*	*%*	*%*	*%*	*gm.*	
49	6/14	25.0	23.0	92.20	90.59	81.4	18.6	28.2	Green, turgid
54	6/19	25.0	20.0	91.39	90.06	80.0	20.0	44.5	" "
58	6/23	21.5	22.0	91.45	88.98	76.2	23.8	54.2	" "
61	6/26	26.5	25.0	90.68	88.36	70.3	29.7	60.3	" "
66	7/1	15.5	17.0	89.18	89.34	62.2	37.8	85.4	Necks beginning to soften
73	7/8	20.0	20.0	91.43	86.26	50.1	49.9	109.4	10 % down
84	7/19	21.0	21.5	90.76	85.85	31.7	68.3	111.8	Mostly down
91	7/26	.....	.....	69.82	84.42	7.6	92.4	92.4	Chlorophyll practically destroyed

was extracted according to the procedure of VICKERY and co-workers (**10**). The amino nitrogen fraction was determined on the water-soluble nitrogen extract by the use of the micro Van Slyke apparatus after the removal of ammonia. Ammonia nitrogen was determined on the water extract mentioned above by proper buffering, then gentle distillation for 10 minutes. The preliminary procedure for amide nitrogen was conducted as recommended by PUCHER and co-workers (**8**); the ammonia was determined as outlined above. In protein nitrogen determinations the plant material was extracted with hot alcohol, then hot water; total nitrogen was determined on the residue (**10**).

The sugars were extracted with hot 80 per cent. ethyl alcohol. After the removal of the alcohol the aqueous solutions were clarified with neutral lead acetate and deleaded with potassium oxalate. The reducing power was determined by the LANE-EYNON method (**1**). The previous alcoholic solution

was also used for determination of non-reducing sugars. The preparation of the aqueous solutions and subsequent reductions were brought about as described above. Inversion was effected by hydrochloric acid at room temperature.

Incineration was made in a muffle furnace over night at a temperature of 550° C. for total ash determinations.

Results and discussion

The results of the investigation are shown in tables I to IV; some of the findings are plotted to illustrate certain relationships more effectively.

TABLE II

THE AMOUNT OF VARIOUS CONSTITUENTS IN THE TOPS AND BULBS AT VARIOUS STAGES OF GROWTH IN GRAMS PER 100 GRAMS OF FRESH WEIGHT

Age	Dry matter	Total nitrogen	Water soluble nitrogen	Amino nitrogen	Ammonia nitrogen	Amide nitrogen	Nitrates	Protein nitrogen	Reducing sugars	Non-reducing sugars	Total ash
days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
					Tops						
49	6.35	0.205	0.053	0.028	0.004	0.003	+	0.900	1.117	0.584	0.810
54	6.89	0.211	0.055	0.032	0.005	0.002	+	0.941	1.256	0.688	0.831
58	6.52	0.190	0.048	0.025	0.005	0.002	+	0.850	1.332	0.612	0.708
61	6.55	0.184	0.047	0.026	0.004	0.001	+	0.835	1.293	0.742	0.701
66	6.73	0.184	0.049	0.032	0.005	0.003	+	0.807	1.409	0.765	0.629
73	4.29	0.115	0.030	0.016	0.003	0.002	+	0.505	0.853	0.402	0.412
84	2.93	0.069	0.022	0.016	0.001	0.001	−	0.297	0.346	0.204	0.341
91	2.29	0.026	0.007	0.003	0.001	0.001	−	0.113	.....	.....	0.654
					Bulbs						
49	1.76	0.029	0.013	0.011	0.001	0.001	+	0.107	0.369	0.555	0.117
54	1.99	0.028	0.014	0.008	0.001	0.001	+	0.101	0.373	0.810	0.114
58	2.62	0.034	0.019	0.011	0.001	0.001	+	0.104	0.406	1.249	0.132
61	3.46	0.044	0.027	0.016	0.002	0.003	+	0.119	0.466	1.715	0.149
66	4.03	0.050	0.034	0.021	0.002	0.004	−	0.117	0.534	2.063	0.164
73	6.86	0.086	0.058	0.031	0.003	0.004	−	0.193	0.742	3.737	0.260
84	9.66	0.145	0.107	0.080	0.006	0.010	−	0.239	0.950	5.345	0.364
91	14.40	0.240	0.194	0.141	0.005	0.019	−	0.299	1.821	7.266	0.600

Chemical changes occurring in the onion during normal growth

The over-all picture of the growth and development of an onion appears to be one in which the initial period of growth is spent in developing a top of great synthesizing powers. The later part of this growth phase is characterized by a rapid gain in dry weight by the plant and a softening of the neck or shank of the top which is due to the fact that new leaves are not being formed. The final period then occurs in which the top gradually ceases photosynthetic activity, and the reserves are hydrolyzed and translocated to the bulb. In this instance, the ratio of the periods was about 2 to 1 respectively.

The reducing and non-reducing sugars in the tops continue to increase until about the 66th day at which time the necks of the tops began to soften (figs. 2, 3). From this point on the sugars are transported to the bulbs to the point of depletion. Since the onion is a sugar plant it is not surprising to find a close correlation between the production of non-reducing sugars in the bulb and the increase in weight of the bulbs (figs. 1, 3).

An accelerated translocation of different forms of nitrogen into the bulbs occurred about the 66th day or in the fifth sample (figs. 4, 5, 6). In the tops the protein represented about 70 per cent. of the total nitrogen at all periods of growth determined. This fraction in the bulb varied from about 58 per cent. at the earliest stage to about 20 per cent. at the final stage. This difference obviously is caused by an accumulation of soluble nitrogen from the

TABLE III

DATA ON THE CONDITION OF THE ONIONS IN CULTURE IN SUNSHINE-DARKNESS AND IN DARKNESS

CONDITIONS	HOURS	GENERAL REMARKS
Sunshine-darkness	0	The tips of the leaves of a few plants were dead and some necks were soft.
	74	About 50% of the necks were soft.
	98	The tops were all flaccid and the outer leaves were yellow; the middle area of tops were still green.
	121	The younger leaves were green and some were turgid. Many of the lower leaves were dead. Seventy % of the necks were soft.
	143	The younger leaves were green and somewhat turgid; the older leaf tips were quite dry and brittle.
Darkness	51	There was little noticeable change.
	93	About 25% of the necks were soft. The tops were becoming more yellow.
	117	About 50% of the necks were soft. The lower older leaves were flaccid and practically all yellow; the younger leaves were quite green and turgid.
	139	Younger leaves were still turgid; about 65% of the necks were soft.

tops. The curves for total and soluble nitrogen in the bulb are quite similar (figs. 4, 5). However, that a bulb which keeps well in storage should have so great an amount of active material, does not appear to be in keeping with the natural laws of economy of biological materials. It seems reasonable, therefore, to assume that if the graph were extended farther to the right there would be evidence to indicate that a synthesis of protein had taken place at the expense of the non-protein fraction.

The onions were pulled on the 85th day and allowed to remain on the ground until the 91st day when they were collected for analysis. Although the tops were brown and somewhat brittle, translocation to the bulbs was not complete (figs. 4, 5, 6). It would seem desirable that this phase of the curing process be brought about under conditions which would favor fairly rapid and continuous processes of dehydration and translocation. It is not unreasonable to suppose that failure to practically deplete the tops of solutes

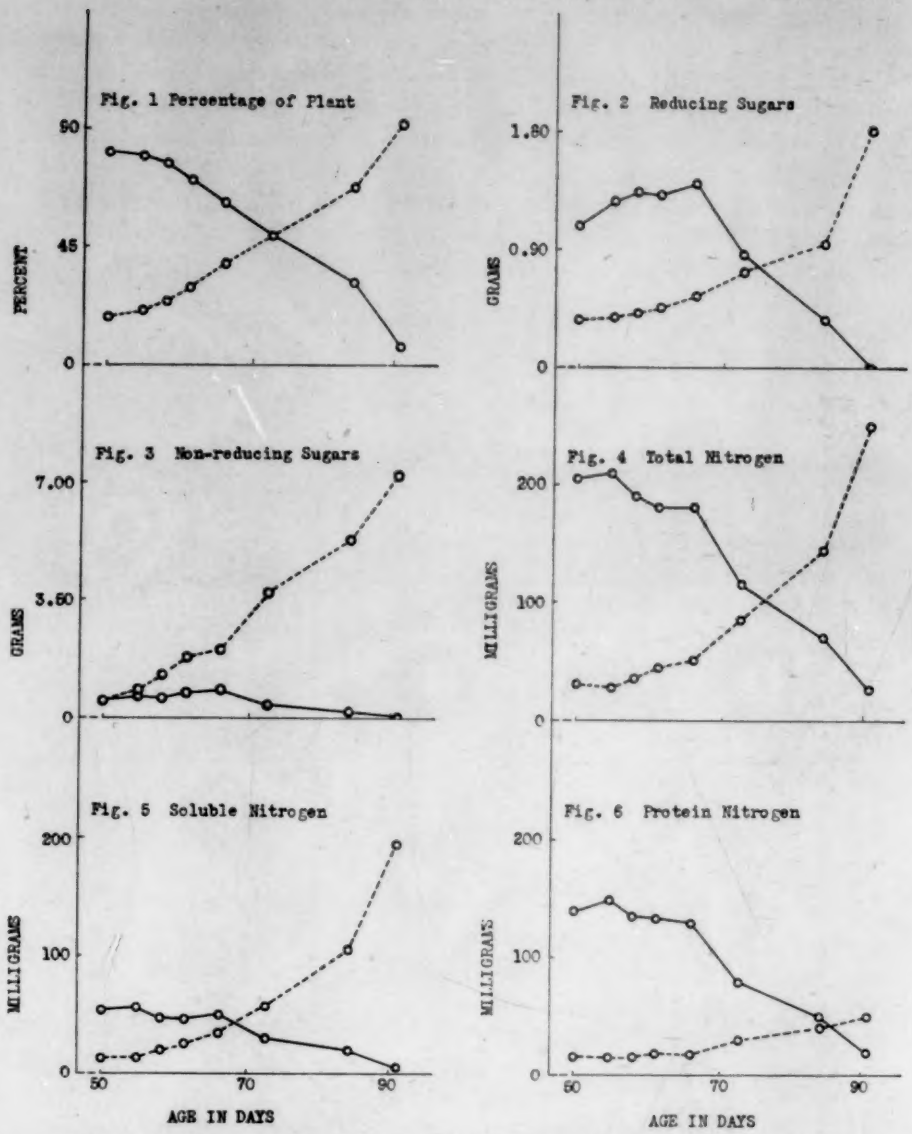
TABLE IV

THE AMOUNT OF THE VARIOUS CONSTITUENTS IN TOPS AND BULBS FROM ONIONS CULTURED IN DISTILLED WATER AND SUBJECTED TO SUNSHINE-DARKNESS AND COMPLETE DARKNESS IN GRAMS PER 100 GRAMS OF FRESH WEIGHT

Hours	Dry matter	Total nitrogen	Water soluble nitrogen	Amino nitrogen	Ammonia nitrogen	Amide nitrogen	Nitrates	Protein nitrogen	Reducing sugars	Non-reducing sugars	Percentage of plant	
											Top	Bulb
Sunshine-darkness												
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%	%
Tops												
0	4.60	0.125	0.032	0.011	0.004	0.001	+	0.093	0.886	0.290	46	.....
74	3.42	0.082	0.028	0.009	0.003	0.001	+	0.056	0.479	0.172	41	.....
98	3.27	0.070	0.030	0.008	0.002	0.001	+	0.044	0.390	0.125	34	.....
121	2.94	0.054	0.020	0.008	0.002	0.001	+	0.033	0.290	0.087	27	.....
143	3.75	0.061	0.024	0.007	0.002	0.001	+	0.024	0.313	0.099	23	.....
Bulbs												
0	7.51	0.095	0.069	0.033	0.006	0.003	–	0.029	0.986	4.242	.....	54
74	6.37	0.099	0.076	0.031	0.006	0.008	–	0.025	0.911	3.298	.....	59
98	8.89	0.153	0.120	0.054	0.009	0.013	–	0.036	1.284	4.538	.....	66
121	10.64	0.212	0.171	0.109	0.012	0.018	–	0.043	1.434	5.116	.....	73
143	10.39	0.211	0.169	0.070	0.011	0.016	–	0.045	1.527	4.918	.....	77
Darkness												
Tops												
0	4.60	0.125	0.032	0.011	0.004	0.001	+	0.093	0.886	0.290	46	.....
51	6.30	0.163	0.050	0.018	0.006	0.001	+	0.107	0.972	0.350	42	.....
93	4.92	0.115	0.050	0.014	0.004	0.004	+	0.029	0.655	0.216	43	.....
117	2.80	0.061	0.024	0.008	0.002	0.001	+	0.036	0.346	0.087	42	.....
139	3.08	0.060	0.023	0.008	0.003	0.001	+	0.036	0.356	0.085	41	.....
Bulbs												
0	7.51	0.095	0.069	0.033	0.006	0.003	–	0.029	0.986	4.242	.....	54
51	8.65	0.123	0.093	0.040	0.006	0.008	–	.....	1.183	4.783	.....	58
93	6.68	0.114	0.089	0.043	0.006	0.010	–	0.029	0.988	3.262	.....	57
117	6.25	0.121	0.095	0.045	0.008	0.009	–	0.028	0.957	3.024	.....	58
139	6.84	0.133	0.105	0.045	0.007	0.012	–	0.029	1.036	3.183	.....	59

in such a manner may have some influence on the synthesis of proteins in the bulbs and on their capacity to keep well in storage.

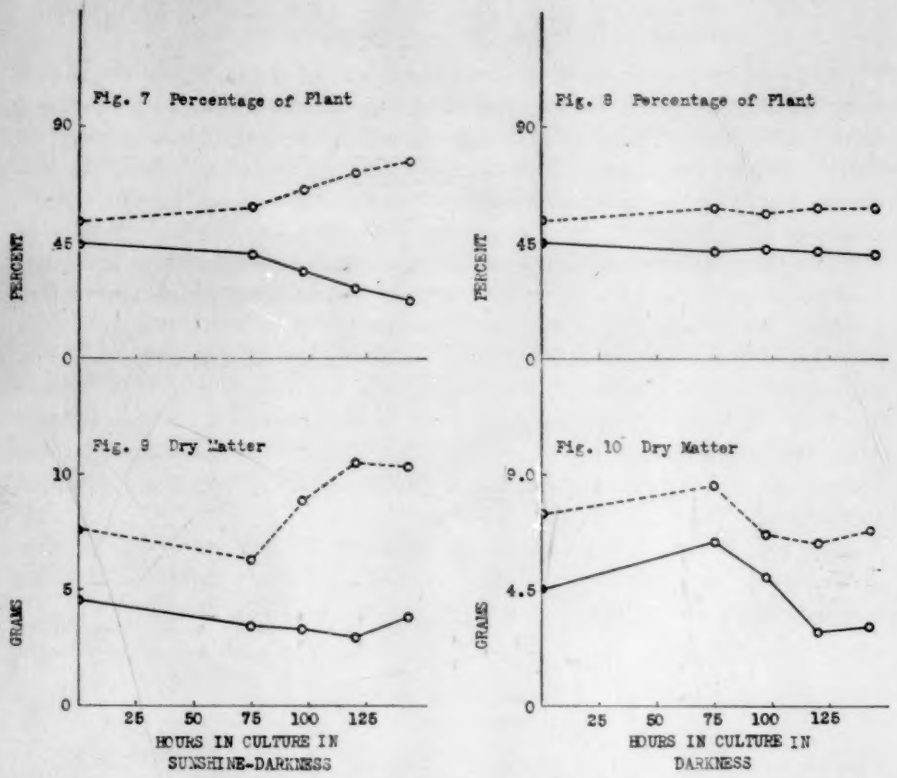
The amino nitrogen in the tops varied irregularly from about 52 to 74



FIGS. 1–6. The percentages of tops (solid line) and bulbs (broken line) and the weights of carbohydrate and nitrogen fractions per 100 grams of fresh whole plant taken at successive stages of growth.

per cent. of the soluble nitrogen; in the bulbs it varied from 53 to 85 per cent. The amide nitrogen fraction in the tops during the season was relatively low and irregular in content; in the bulbs, however, this fraction

increased throughout the growing period and was especially significant during the later period, during which time there was a gradual breakdown of chlorophyll. At this stage of development the bulb contains a considerable amount of soluble compounds from the tops. Thus the production of ammonia may occur by oxidative deamination of the amino acids with the ultimate formation of amides (**7**). Since the levels of ammonia and amide nitrogen were relatively low prior to this stage, there seems little doubt that amino acids from hydrolyzed proteins of the tops serve as a source of the ammonia (**3, 6, 11**).



FIGS. 7–10. The percentages of tops (solid line) and bulbs (broken line) and the weights of the dry matter per 100 grams of fresh whole plant cultured in distilled water in sunshine-darkness, and in darkness respectively.

Nitrates were present in all tops except the last two samples, but they were present in the bulbs only through the first four stages of growth or the 61st day. It seems therefore that as soon as new leaves cease to be produced, nitrates no longer occur in the bulbs. This would indicate either that the uptake of nitrates thereafter is not so great but what complete reduction occurs in the bulbs or that the capacity for reduction in the bulbs has increased. The presence of nitrates in the tops would perhaps indicate an inherent inability to reduce them. If the ammonia formed is converted to

an amide, the enhancement of the latter fraction may also be accounted for to some extent from the nitrates.

Although there are many factors affecting the absorption of inorganic nitrogen by plants, it is interesting in view of our findings to note the observations of some others. In many species, CAMPBELL (**2**) found the highest percentage of nitrate nitrogen to exist just prior to the blossom stage; in some plants it completely disappeared at full maturity. CULPEPPER and CALDWELL (**4**) are of the opinion that high nitrate absorption is a function definitely limited to the early vegetative stages.

CHEMICAL CHANGES UNDER ABNORMAL CONDITIONS

The over-all picture of the general conditions of the plants is best shown in figures 7 and 8 which disclose that a rapid translocation from tops to bulbs began after about 75 hours in the greenhouse, whereas plants exposed to complete darkness showed little translocation during 139 hours. In the greenhouse both the tops and bulbs lost weight rather steadily for about 75 hours at which time enforced maturity took place and the bulbs began to increase in weight at the expense of the tops (fig. 9). In darkness both tops and bulbs increased in weight for about 50 hours, after which respiration assumed the dominant rôle and both portions of the plant lost weight at a somewhat similar rate (figs. 8 and 10). In general, reducing and non-reducing sugars and the nitrogen fractions follow the trends shown in figures 9 and 10. Translocation of nitrogen into the bulb was slow in darkness and after 139 hours was relatively incomplete. Under these conditions, as was the case during normal growth, ammonia and amide nitrogen accumulated in the bulb.

The results obtained from onions cultured in distilled water in alternate sunshine and darkness and in continuous darkness are compared with those obtained from onions produced normally in the following chart:

CONDITIONS	NITROGEN		SOLUBLE SUGARS	
	TOP	BULB	TOP	BULB
Sunshine-darkness	Normal	Normal	Decrease	Normal
Darkness	Normal	Normal	Decrease	Decrease

The principal chemical change, therefore, effected by these conditions was a decrease in the soluble sugars which was more extensive in complete darkness.

Summary and conclusion

Analyses of the tops and bulbs of the Ebenezer onion were made on onions at various stages of growth in soil and on onions cultured in distilled water in sunshine-darkness and in darkness.

In general, a consideration of seasonal trends of some of the constituents of the Ebenezer onion indicates that the normal growth cycle may be divided

into two parts. The first, consisting of about two-thirds of the total growing period, is spent in developing a top of great synthesizing powers, and is marked in the later stages by a rapid gain in dry weight and a softening of the neck or shank of the plant. The second period is characterized by a gradual suspension of photosynthetic activity and by a more or less complete removal of solutes to the bulb. As a result of these physiological processes it may be generally stated that in the tops the content of total ash and of all the nitrogen fractions, determined quantitatively, decreased with maturity accompanied by an increase of soluble sugars until signs of maturity develop; in the bulbs the content of all constituents increased.

1. The chief points of significance for plants grown in soil are that the amides accumulate in the bulbs, especially during the second period; about 80 per cent. of the total nitrogen of the bulb at maturity is soluble and reduction of nitrates appears to take place in the bulb.

2. In comparison with the above, the chief changes which occurred in the onions in water culture were that transporation of solutes from the tops to the bulbs occurred very slowly during 139 hours in complete darkness and that complete darkness resulted in a greater loss of the soluble carbohydrates than did sunshine-darkness.

The author wishes to record his indebtedness to Dr. W. G. Colby and Mr. Hrant M. Yegian of the Department of Agronomy for the supply of onions and for helpful criticisms.

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SUGARS IN THE ROOT OF THE CARROT

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Introduction

There is a lack of agreement in the literature as to whether or not fructose occurs in the roots of the carrot. Apparently the reducing sugar is thought by many to be composed largely or entirely of glucose, and that little or no fructose is present.

Sucrose and glucose have been reported by Dietrich (**5**), Rittenhausen (**5**), Platenius (**8, 9**), Barnes (**1**), and Werner (**14**). Sucrose was identified by Schmidt (**5**) and glucose by Busholt (**5**). Sucrose and reducing sugar were reported by Myers and Kroll (**5**), and sucrose and invert sugar by Kristofferson (**5**) and by Wehmer (**13**). When reducing sugars are reported by these workers, no conclusion can be drawn as to what the workers considered the sugars to be. Kristofferson (**5**) and Wehmer (**13**) are the only ones to report the reducing sugars as being "invert sugar," and thereby indirectly to admit the presence of fructose. Analyses of two commercial varieties of carrots were made in the present study to determine the possible presence and amounts of fructose.

Materials and methods

Carrots of the Danvers and the Imperator varieties were used. Lot 1 of table I was of the Danvers variety; all of the remaining lots were Imperator. The samples were preserved by boiling in alcohol. Lot 1 was brought from the field on August 16, 1943, at which time the roots had an average weight of 143 grams. The field had been planted in September, 1942. Commercial harvesting had been in progress previous to the date of collection and a large number of plants had developed seed stalks. Sample 1A was preserved immediately after the lot was brought from the field, whereas sample 1B was held at room temperature (about 24° C.) for 2 days before the sample was preserved. Lot 2 was collected on December 22, 1942, from a field that had been planted in August, 1942; the roots had an average weight of 170 grams. Commercial harvesting had not been started in this field at the time of collection, but the carrots used were easily large enough for the market. Lot 2 was held at 3° C. and 85 per cent. relative humidity for 15 days before the sample was preserved. Lots 3 to 6 were collected from one field at the time of day indicated in table I, all on August 30, 1943. The field had been planted in September, 1942. As in the field from which lot 1 was taken, commercial harvesting had been carried on for several weeks before the collection date, and many plants had produced seed stalks, though these were omitted from the samples. The average weight of the roots of lots 3 to 6 was 151 grams.

Qualitative tests for fructose were made by means of the Flückiger reaction [copper tartrate test (**4, 7, 11, 12**)] and the osazone test. The osazone

test was also used to test for the presence of maltose. Quantitative sugar determinations were made by means of the methods of QUISUMBING and THOMAS (10), BEST, PETERSON, and SELL (2), and JACKSON and MATHEWS (3, 6, 7). The increase in reducing power resulting from hydrochloric acid hydrolysis was taken to be due to sucrose. Glucose was taken to be the difference between total reducing sugars and fructose. Commercial baker's yeast was used for elimination of fermentable substances.

Results

The Flückiger reaction gave positive results for fructose. While the osazone test was positive for fructose in time of formation of precipitate when comparison was made with solutions of pure fructose and pure glucose,

TABLE I

SUGAR AND WATER CONTENT OF CARROT ROOTS; GIVEN AS PERCENTAGE OF FRESH WEIGHT

LOT	PORTION OF ROOT	TIME OF DAY	FRUCTOSE	GLUCOSE	SUCROSE	TOTAL SUGARS	FRUCTOSE: GLUCOSE	WATER
			%	%	%	%	*Ratio*	%
1A	Composite	1 P.M.	0.25	1.21	5.05	5.51	1.19:1	96.9
1B	Composite	.....	1.91	1.95	3.55	7.41	0.98	83.3
2	Composite	.....	1.70	1.57	3.63	6.90	1.08	87.3
3	Xylem	7 A.M.	0.22	0.39	4.98	5.57	0.56	86.4
3	Phloem	7 A.M.	0.13	0.27	4.91	5.31	0.48	85.0
4	Xylem	11 A.M.	0.29	0.44	4.44	5.17	0.66	87.0
4	Phloem	11 A.M.	0.31	0.39	4.39	5.09	0.80	85.8
5	Xylem	3 P.M.	0.51	0.59	3.46	4.74	0.86	88.1
5	Phloem	3 P.M.	0.25	0.26	4.71	5.22	0.96	86.3
6	Xylem	7 P.M.	0.34	0.23	5.16	5.73	1.48	87.1
6	Phloem	7 P.M.	0.21	0.23	5.14	5.58	0.91	85.4

the results of this test are not taken to be conclusive. The osazone test did not give any indication of the presence of maltose. The presence of sucrose in the solutions made the resorcinol test for fructose unreliable. The quantitative method of JACKSON and MATHEWS (6) for the determination of fructose gave positive results (table I).

Baker's yeast was allowed to act on an extract of carrot tissue. The solution was prepared by extracting finely ground tissue with 80 per cent. alcohol, removing the alcohol from a portion, and taking up the soluble solids in water. After the yeast had acted on the filtered solution for 18 hours at 22° C. no measurable reducing substances remained. This was true both when the fermentation was carried out before and after acid hydrolysis for the determination of total sugars. It is therefore to be assumed that the reduction of copper which occurred in the procedures that were used was brought about by fermentable sugars, and not by either pentoses or some non-sugar reducing substance or substances.

Results show that the Danvers and the Imperator varieties contain ap-

preciable amounts of fructose (table I). There was no indication of any material difference in the total sugar content, or in any of the sugar fractions, between the xylem and the phloem. The increase in the total sugar content in lot 1B over 1A during the 2-day holding period was brought about by the hydrolysis of polysaccharides, presumably mainly starch, since starch is known to be present. No data are available on the samples under discussion in this paper, but in similar material it was found that the increase in total sugars during holding was accompanied by a corresponding decrease in the acid hydrolyzable polysaccharides. During the same 2-day holding period the proportion of reducing sugars increased from 8 to 52 per cent. of the total sugars. The increase in the reducing sugars was brought about in part by the inversion of sucrose and in part by the hydrolysis of polysaccharides.

Discussion and summary

Data which have been obtained by the method of JACKSON and MATHEWS (**6**) indicate that fructose occurs in the roots of the Danvers and the Imperator varieties of carrot. The results obtained by this method are supported by those obtained by the Flückiger reaction (**4, 7, 11, 12**), and by the osazone test to the extent that this method can be considered reliable.

Although the osazone test gave negative results for maltose, it is reasonable to suppose that a very small amount was present since it is known to occur as an intermediate product between starch and glucose.

The presence of sucrose and glucose in the carrot root is generally accepted, but there is disagreement with respect to fructose. The more recent literature has indicated that fructose is absent, or present in only minute quantities, and that the reducing sugar is composed almost entirely of glucose. Evidence is offered in the present paper which indicates that fructose occurs in quantities approaching one-half of the total reducing sugars present. If maltose was present it occurred in quantities too small to be detected by the osazone test.

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LEAF ANALYSIS AS AN INDICATOR OF POTASSIUM REQUIREMENT OF CANE FRUITS[1]

HARRY E. CLARK AND WILBUR L. POWERS

(WITH ONE FIGURE)

In the past, several methods have been used to determine the adequacy of the potassium supplying power of different soils. Recently several workers have analyzed plant leaves as a possible index to the level of potassium used or needed for optimum growth. It is to be desired that standards for potassium content of normal leaves may be developed. The study herein reported was undertaken to determine the value of leaf analyses as compared to soil and fruit for indicating potassium requirements, and with the hope of establishing the normal and critical potassium level for the foliage of certain crop plants.

WALLACE (**20**) and also LOEHWING (**10**) reported that liming soil might increase potash hunger, interfere with carbohydrate storage, or induce chlorosis by making the sap too alkaline to maintain iron in solution. LIPMAN, BLAIR, and PRINCE (**9**) found the potash in corn stalks to vary from 0.346 to 3.209 per cent. depending upon the fertilizer used. Application of potash increased and liming decreased potash in stalks, but yields were not reported uniformly. VAN SLYKE (**19**) reported the potassium contents of plant parts including leaves. THOMAS and MACK (**15, 16**) have described the salient features of foliar diagnosis. DARROW and MAGNESS (**3**) reported that mulching increased available potash and the content of this element in raspberry leaves.

WARNE (**21**) found the potassium content of "scorched" or potassium-deficient apple leaves to be lower than that in normal leaves. REUTHER and BOYNTON (**13**) found the lower limit of potassium content in the scorch-free leaves of McIntosh apple trees to be about one per cent., although in certain instances leaves containing only 0.75 per cent. were free of potash deficiency symptoms. According to CULLINAN and WAUGH (**2**) the potassium content of peach leaves showing acute deficiency symptoms is less than one per cent. on a dry weight basis. Leaves with a potassium content of 1.0 to 1.25 per cent. did not show marked symptoms, yet trees from which they were taken could be expected to respond to applications of potassium. If the leaf content was between 1.5 and 2.0 per cent., tree response to added potassium was erratic. Although the extreme range of potassium content of peach leaves in June and July was from 0.6 to 3.4 per cent., the average was about 2.0 per cent.

Potash deficiency symptoms of leaves have been described and pictured by ECKSTEIN, BRUNO, and TURRENTINE (**4**) and by others (**6**). A review of the literature shows that a better knowledge of the potassium content of

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normal leaves and leaves showing potassium deficiency symptoms is needed. Many studies have been made which show the increase in potassium content of plants with potassium fertilization. Among these studies are those by JANSSEN and BARTHOLOMEW (7), HARTT (5), VANDECAVEYE (18), and STUBBLEFIELD and DE TURK (14). In several of these studies, "luxury" consumption of the element was indicated at the higher potassium levels.

Materials and methods

Field plot fertilizer trials were initiated with Cuthbert raspberries, boysenberries, and black raspberries. Soils used in this study were Powell and

TABLE I

NEUBAUER TESTS WITH POWELL SILT LOAM SOIL

TREATMENT AND N-P-K RATIO	DEPTH	K_2O PER 100 GM. SOIL	K_2O PER ACRE (2,000,000 lb.)
Lb. per acre	*in.*	*mg.*	*lb.*
Red raspberry plots:			
Check	0–8	11.61	232
	8–20	0.72	14
	20–36	1.00	20
500 (5–20–10)	0–8	12.80	256
	8–20	1.20	24
	20–36	—	—
1000 (5–20–10)	0–8	30.15	603
	8–20	6.80	136
	20–36	0.84	17
1500 (5–20–10)	0–8	31.60	632
	8–20	6.42	128
	20–36	4.80	96
Boysenberry plots:			
500 (5–20–0)	0–8	3.54	71
	8–20	—	—
	20–36	—	—
500 (5–20–20)	0–8	5.08	102
	8–20	4.02	80
	20–36	3.65	73
Wooded virgin soil, nearby	0–10	21.70	435
	10–30	11.92	238
Soil cropped 50 yrs, nearby	0–10	4.32	86
	10–30	5.26	105

Olympic silt loams. The land had been cropped to grain for 50 years or more before the berries were planted. The first leaf below the growing tip was collected from 50 canes at harvest time for analysis.

Powell silt loam consists of 10 to 15 inches of friable brown to light brown silt loam on a slightly mottled gray to yellowish brown silt loam, firmer horizon at 12 to 30 inches depth. Below this accumulation horizon is a lighter yellowish-brown material with water-worn gravels in places at 6 feet or more in depth. The surface soil of Powell silt loam at the boysenberry check plot

was found to have a pH value of 5.15, total nitrogen content of 3,680 pounds, and available phosphate of 45 pounds, in 2,000,000.

Olympic silt loam from the black raspberry check plot to a depth of 10 to 12 inches is friable, reddish-brown silt loam, with iron concretions. Below this is brighter reddish-brown, compact, silty clay loam over basaltic tufa and lava rock at 3 to 6 feet or more. The pH was 5.2 and the available potassium 32, total nitrogen 2,440, and available phosphate 40 pounds in 2,000,000.

NEUBAUER tests were made on soil samples taken from the fertilizer trial plots and from adjoining fields (table I), according to the procedure outlined by THORNTON (**17**). Leaf samples were dried in an electric oven at 70° C., and ground to pass a 40-mesh screen. The potassium content of the ground leaf material was determined by ashing a 5-gram, oven-dry sample of the ground leaves at 600° C. The residue was taken up in dilute nitric acid and filtered, washed several times with small quantities of hot water, the filtrate cooled, and its potassium content determined according to the procedure of Wilcox (**22**).

The water soluble potassium of soils was extracted according to the procedure outlined by NOGGLE and WYND (**11**). Normal ammonium acetate was used in leaching the soil on a Buchner funnel in the extraction of replaceable potassium.

Results

SOIL POTASSIUM AVAILABILITY

Data from NEUBAUER tests show that the amount of available potassium present in loam soil after two years of fertilizer treatment varies according to the treatment (table I). The available potassium tends to increase with rate of application. This increase is found especially in the red raspberry plots. Increase in available potassium with the heavy application extends below plow depth or into second soil horizon.

An analysis of the Olympic silt loam soil where the proposed fertilizer trial with black raspberries was to be located supplied the data shown in table II. Virgin soil nearby was found to have 108 pounds per acre of NEUBAUER potash while adjacent old grain land had only 59 pounds in 2,000,000 of surface soil. Another test with Olympic silt loam soil sample in the proposed trial area yielded only 48 pounds in 2,000,000. Analysis of the two soils, Powell silt loam and Olympic silt loam (tables I and II) show that these soils are low in available potassium until they have been treated with fertilizer.

POTASSIUM IN RED AND BLACK RASPBERRY LEAVES

The average percentage of potassium, oven-dry basis, in red raspberry leaves and fruits according to the various fertilizer treatments is given in table III. There is an erratic increase in the potassium content of the leaves when a fertilizer rate of 500, 1000, or 1500 pounds is applied as compared

TABLE II

POTASSIUM CONTENT OF OLYMPIC SILT LOAM SOIL

SAMPLE	DEPTH	NEUBAUER POTASSIUM, K_2O PER 100 GM. SOIL	WATER-SOLUBLE POTASSIUM, K_2O PER 100 GM. SOIL	REPLACEABLE POTASSIUM, K_2O PER 100 GM. SOIL
	in.	*mg.*	*mg.*	*mg.*
Check plot	0–7	0.029	1.60	12.65
	10–24	0.05	0.38	10.23

to the average of the check plots. Some seasonal variation is indicated. Increase in potassium in fruits from use of potassic fertilizer appears to be less consistently realized.

Before starting a field fertilizer trial with black raspberries, leaves showing potash deficiency symptoms and apparently normal leaves were analyzed. The discolored, potassium-deficient leaves contained approximately one-third as much potassium as the normal green leaves (figure 1). Application of potassium fertilizer increased the cover crop growth and with heavily fertilized plots growth is considerably greater the first season. Size and yield of fruit was usually increased the second year of the experiment (table IV). Net profit was highest when 500 pounds of 5–20–20 fertilizer was applied.

The average percentage of potassium in black raspberry leaves is given

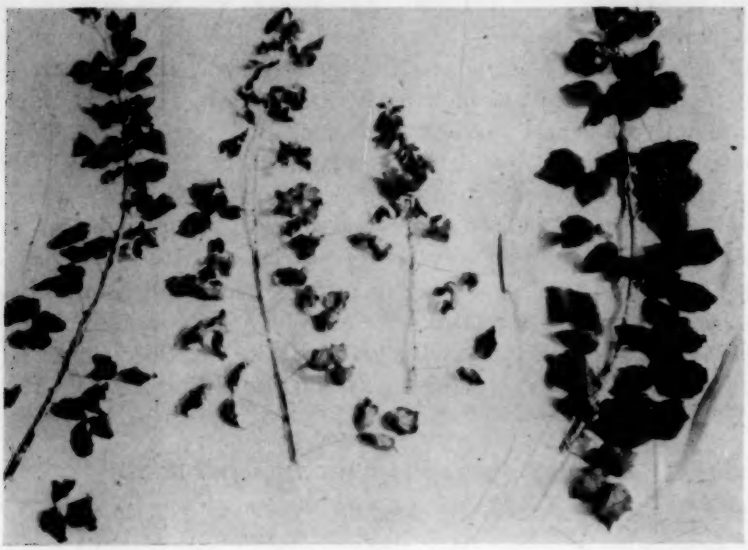

FIG. 1. "Scorched" black raspberry leaves from untreated plot (left) and from 4–12–12 plot (right).

TABLE III

POTASSIUM CONTENT OF RED RASPBERRY LEAVES AND FRUITS

Plot	Treatment and N-P-K ratio	Potassium in cane fruit				
		Leaves			Fruits	
		1939	1940	1941	1941	1942
	Lb. per acre	%	%	%	%	%
1	Check	.....	0.75	0.85	.....	0.476
3	500 (5–20–10)	0.71	0.87	0.87	0.918	0.635
6	100 (5–20–10)	1.26	1.02	1.15	.....	.....
7	Check	0.97	0.94	0.95	0.406	0.461
8	500 (5–20–10)	1.23	1.10	1.20	.....	.....
9	1500 (5–20–10)	1.02	0.95	1.32	0.610	0.512
10	10 T. manure	1.12	.....	1.13	.....	.....
11	Check	1.22	0.97	1.22	0.612	0.445
2	(–K) (5–20–0)	.....	.....	.....	.....	0.524
Average	Checks	1.09	0.88	1.01	0.51	0.46

in table V for the leaves sampled in 1940 and 1941, and for fruits in 1941 and 1942. The potassium content of the leaves tended to increase on fertilized plots while that of fruits was little affected. Berry pickers preferred to harvest plots treated with a heavy potash application as the fruits were found to be larger.

The variation in potassium content from year to year may be due to dif-

TABLE IV

RESPONSE OF BLACK RASPBERRIES TO FERTILIZER ON OLYMPIC SILT LOAM SOIL*

Plot	Treatment or N-P-K ratio	No. fruits per lb., 1941	Yield			Average 3 years	Increment	Net profit or loss from treatments
			1940	1941	1942			
			tons	*tons*	*tons*	*tons*	*tons*	*dollars*
1	Check	.....	0.69	0.41	0.60	0.57	.....	.....
2	5–20–0	398	0.67	0.46	0.40	0.51	– 0.09	– 20.70
3	5–0–10	378	0.73	0.62	1.15	0.89	0.29	47.01
4	Check	407	0.70	0.38	0.40	0.49	.....	.....
5	0–20–10	376	0.76	0.45	1.13	0.78	0.18	27.01
6	5–20–15	381	0.77	0.59	1.44	0.93	0.33	51.80
7	Check	.....	0.66	0.32	0.44	0.47	.....	.....
8	5–20–10	458	0.45	0.50	0.83	0.59	– 0.01	– 7.82†
9	5–20–20	333	1.13	0.82	1.70	1.22	0.62	102.54
10	Check	.....	1.19	0.52	0.89	0.87	.....	.....
1, 4, 7, and 10 checks, average			0.81	0.41	0.58	0.60	.....	.....

* Black raspberries valued at 9¢/lb. Basic rate of 500 lbs. 5–20–10, using calcium nitrate, treble superphosphate, and potassium chloride at 2.35¢, 2.15¢, and 1.9¢ per pound, respectively.

† This plot contained some unthrifty hills which may be reflected in number of fruits per pound.

TABLE V

POTASSIUM CONTENT OF BLACK RASPBERRY LEAVES AND FRUITS

PLOT	TREATMENT AND N-P-K RATIO	POTASSIUM PRESENT			
		LEAVES		FRUITS	
		1940	1941	1941	1942
	Lb. per acre	%	%	%	%
4	Check	0.20	0.33	.....	.....
6	500 (5–20–15)	0.41	0.55	0.11	0.42
7	Check	0.20	0.32	0.16	0.36
8	500 (5–20–10)	0.37	0.42	0.13	0.31
9	500 (5–20–20)	0.50	0.68	0.19	0.46
10	Check	0.24	0.44	.....	.....

ferences in climatic, soil, and growing conditions. This yearly variation is similar to the work reported by LILLELAND and BROWN (**8**). Leaves reflect the potassium treatment more accurately than the black raspberry fruits.

POTASSIUM IN BOYSENBERRY LEAVES

Boysenberry leaves showing bronzed or brown-cupped edges and, in most cases, interior brown spots (indicating potash deficiency) were found to be low in potassium as compared to leaves grown on plots receiving a complete fertilizer. The observation of potash-deficiency symptoms on the leaves found by chemical analysis to be low in potassium, led to the initiation of a potash variation trial on boysenberries.

The potash variation trial, in which only the amount of applied potash was varied, was divided into two parts in order to study the effect of applying the fertilizer in furrows near the plants in comparison with broadcasting the fertilizer on each side of the row to be used in obtaining the yield data.

TABLE VI

FERTILIZER TRIAL WITH BOYSENBERRIES ON POWELL SILT LOAM SOIL

TREATMENT AND RATIO	YIELD PER ACRE				AVE. NET GAIN	
	1940	1941	1942	3-YR. AVE. YIELD		NET PROFIT FROM TREATMENT*
Lb. per acre	*tons*	*tons*	*tons*	*tons*	*tons*	*dollars*
Fertilizer in furrows:						
Check	5.20	3.35	2.97	3.84	.....	.....
5–20–10	6.93	4.19	3.23	4.78	−0.9	131.61
Fertilizer broadcast:						
Check	6.55	3.62	3.24	4.47	.....	.....
5–20–10	6.27	3.57	3.09	4.31	−0.16	−33.39
Combination of both:						
Check	5.85	3.48	3.11	4.15	.....	.....
5–20–10	6.61	3.88	3.16	4.55	0.40	48.88

* Berries valued at 7.5¢/lb.

TABLE VII

BOYSENBERRY CANE MEASUREMENTS

PLOT	TREATMENT AND N-P-K RATIO	AVERAGE DIAMETER, 100 CANES	PROBABLY ERROR
	Lb. per acre	*1/32 in.*	*1/32 in.*
1	Check	13.98	0.060
2	500 (5–20–10)	14.29	0.057
3	500 (5–20–15)	14.40	0.057
4	Check	14.00	0.054
5	500 (5–20–20)	14.67	0.066
6	500 (5–20–0)	14.51	0.065

According to the data in table VI, the application of fertilizer in furrows near each side of the row is more effective and economical than broadcasting. Where fertilizer was broadcast the crop cover grew to a more even height and may have competed more effectively with the cane fruit for nutrients supplied. Boysenberry cane measurements were made in 1941 on the potash variation trial plots. An increase in stem diameter resulted from the fertilizer treatment which is mathematically significant in some cases.

The average percentage of potassium in boysenberry leaves from some of the fertilizer trials with major elements and those from the potash variation trial are given in table VIII. The potassium content of the leaves for plots treated with increasing amounts of fertilizer shows that a complete fertilizer tends to increase the potassium content of leaves. In the case of the heavy application of 5–10–20 fertilizer phosphate may tend to depress leaf potassium, as CHAPMAN (1) has suggested.

Results of leaf analysis, NEUBAUER tests, and crop response all show similar trends. The result of fertilizer application may be observed in both leaf and soil analyses as well as in increased production. Leaf analysis is a definite indicator of the amount of potassium demand by the plant at least until

TABLE VIII

POTASSIUM CONTENT OF BOYSENBERRY LEAVES

PLOT	TREATMENT AND N-P-K RATIO	POTASSIUM CONTENT OF LEAVES, 1941
	Lb. per acre	*%*
Major elements:		
3	500 (5–20–10)	1.02
5	Check	0.86
7	1000 (5–20–10)	1.02
8	1500 (5–20–10)	1.01
9	Check	0.84
Potash variation:		
1	Check	0.62
2	500 (5–20–10)	0.56
3	500 (5–20–15)	0.64
4	Check	0.57
5	500 (5–20–20)	0.60
6	500 (5–20–0)	0.51

TABLE IX

POTASSIUM CONTENT OF BOYSENBERRY FRUIT

PLOT	TREATMENT AND N-P-K RATIO	POTASH CONTENT	
		1941	1942
Potash variation:	*Lb. per acre*	%	%
2	500 (5–20–10)	.....	0.52
3	500 (5–20–15)	0.12	0.46
4	Check	0.11	0.40
5	500 (5–20–20)	0.36	0.63
6	500 (5–20–0)	0.04	0.36

"luxury consumption" begins. The data in table IX indicate that the potassium content of the fruit tended to increase with the use of potassium as a fertilizer particularly in 1942. Seasonal variation may be due to difference in stage of maturity.

RELATION BETWEEN POTASSIUM APPLICATION AND INVERT SUGAR CONTENT

The black raspberries from the 1941 crop tasted sweeter, according to many who judged them, when they had been fertilized liberally with potassium. To check this, determination of invert sugar was made in our Soils Laboratories and data are reported in table X. Samples represent several cups of fruit in each case. There seems to be more sugar in the fruit from the better yielding plots and in those grown with an adequate potassium supply than in the others. There is no evident reason to advance for the difference in sugar content of the two checks. Phosphate without potash may, however, depress potassium absorption and sugar formation. Small increases in vitamin C content of berries were found from use of potassium according to determinations made in the laboratory.

TABLE X

POTASSIUM FERTILIZATION IN RELATION TO INVERT SUGAR IN BERRIES

	FERTILIZER TREATMENT		INVERT SUGAR
			%
Black raspberries—	None		2.72
1941 pack	Low potassium	(N + P)	0.92
	Medium potassium	"	2.64
	High potassium	"	3.20
Fresh Boysenberries—	None		3.36
1942 crop, fresh	No potassium	(N + P)	2.20
	Low potassium	"	2.56
	Medium potassium	"	3.96
	High potassium	"	3.00
Fresh red raspberries—			
1942 crop, Major Plot 1	None		2.72
Major Plot 7	None		1.36
Major Plot 2	5–20–0		0.88
Major Plot 3	5–20–10 Lbs./Acre		3.68

Discussion

Standards are needed as to the various mineral nutrients in normal leaves in order to make use of leaf analysis as an index to fertilizer needs. THOMAS and MACK (**15**) studied the nitrogen, phosphorus, and potassium contents of leaves of the same physiological age. They found that the addition of each of the dominant nutrient elements to the soil increased the content of the element in the leaves sampled whether the element was applied alone or in combination with one or both of the other major fertilizer constituents. Utilization of any nutrient, however, is greatly influenced by the presence of other nutrients in the fertilizer.

PRATT (**12**) analyzed red raspberry leaves from plants grown under a mulch and those with regular cultivation. The leaves from mulched plants contained 2.39 per cent. potash and those under cultivation contained only 0.86 per cent. potash. Apparently potash was leached from the organic mulch and absorbed by the raspberry plants. Where potassium deficiency symptoms have been observed in black raspberry and boysenberry acreages in Oregon, the leaves turn brown, or appear "scorched" near the edges. Later the entire leaf becomes cupped, dies, and abscission follows. The readily available potassium in the soil on which black raspberries were grown was found to be low. Preliminary leaf analyses before plots were established revealed low percentages of potassium in the affected leaves as compared to normal leaves. Leaf analyses, while requiring less time than needed for NEUBAUER cultures and analyses, appear to give less consistent results.

Summary

Cane fruit leaves and fruits from three experiment plots on Powell and Olympic silt loams supplied with varying amounts of fertilizer were analyzed for potassium content. Use of potassium salts increased available potassium in soils low in this nutrient; caused increased growth of cover crop; larger cane diameter; increased potassium in foliage; and was related to larger, firmer berries and greater yields from black raspberries and boysenberries.

The critical level of potassium in black raspberry and boysenberry leaves was found to be 0.20 and 0.50 per cent., respectively, under conditions for which the leaf composition was studied. Thrifty black raspberry leaves had 0.47, boysenberry leaves 1.00, and red raspberry leaves 1.20 per cent. potassium. The NEUBAUER test is longer, yet may give somewhat more consistent indication of potassium used than leaf analysis. Application of potassium carriers appears to increase the potash content of the leaves more than it does that of the fruit.

The authors wish to thank DR. J. R. HAAG for cooperation in determination of vitamin C, MRS. P. C. PANG for determination of potassium in berries, and PROFESSOR THOMAS ONSDORFF for determination of fruits per pound.

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ABSORPTION OF HYDROCYANIC ACID BY VALENCIA-ORANGE FRUITS AND LEAVES FUMIGATED AT ATMOSPHERIC PRESSURE AND AT PARTIAL VACUUM[1]

E. T. Bartholomew, Walton B. Sinclair, and D. L. Lindgren

(WITH FOUR FIGURES)

Fumigation of enclosures and commodities at atmospheric pressure, for the killing of pest infestations, has been practiced widely for many years. According to Monro (**6**) and others, the first fumigations at reduced pressure were made simultaneously about 30 years ago, by Mackie (**5**) in the Philippines, and by Sasscer and Hawkins (**9**) in the United States. It appears, however, that such fumigations were attempted at least as early as 1892.[2] Fumigations at reduced pressures are generally, but erroneously, called "vacuum" instead of "partial vacuum" fumigations. Because of the common usage, the two terms will be used interchangeably in the present paper to designate fumigations made at reduced pressure, in contrast to those made at atmospheric pressure.

Two kinds of vacuum fumigation have been used—namely, the sustained and the dissipated. In sustained-vacuum fumigation, air pressure is reduced the desired amount, the fumigant is allowed to enter the chamber, and the partial vacuum is maintained during the entire fumigation period. In dissipated-vacuum fumigation, the pressure is first reduced, and then the vacuum or partial vacuum is dissipated. The fumigant is introduced during or shortly before dissipation. Atmospheric pressure is maintained during the fumigation period. At the end of the fumigation period, the air and fumigant are removed from the chamber, and at least partially from the commodity, by again reducing the pressure, a process which is sometimes repeated two or three times.

The early history of fumigation has been summarized by Essig (**4**) and Quayle (**8**). In a recent article, Monro (**6**) discusses the basic theories of fumigation, with special reference to the penetration of gases into the commodities; he also raises a question as to the validity of the use of the term "vacuum" fumigation.

Fumigants such as hydrocyanic acid, methyl bromide, carbon disulphide,

[1] Paper no. 517, University of California Citrus Experiment Station, Riverside, California.

[2] In a letter to the senior author, dated February 24, 1944, the late Dr. E. B. Mackie, then chief of the Bureau of Entomology and Plant Quarantine of California, stated that fumigations at reduced pressure were attempted, and in some cases patents were applied for, by a Swiss in Paris in 1892; a Frenchman in 1910; and by a Hollander in Java in 1915; and that there is also a German record of about this last date. Exact citations are not given in the letter, and inasmuch as it is not intended to present a historical discussion of fumigation in this paper, no attempt has been made to trace these citations.

and ethylene oxide–carbon dioxide are in common use. Only hydrocyanic acid (HCN) was used in the present experiments.

Other workers have attempted to determine the amount of fumigant absorbed by a commodity by measuring the amount of free gas in the chamber at the end of the fumigation period. In the present experiments, as in those previously reported from this laboratory (**1, 2, 10**), the amount of fumigant absorbed by the material treated has been determined directly by measuring the amount recovered from the fumigated material. This is by far the more accurate of the two methods and is, in fact, the only accurate method when the volume of the commodity in the fumigation chamber is small.

The data presented in this paper are not related to pest mortality, but are the results of experiments performed primarily to determine the effects of various factors on the relative amounts of HCN that could be recovered from fumigated citrus fruits and leaves. The experiments were performed to determine: (a) the effects, on fruits, of exposure to HCN for different lengths of time, at atmospheric pressure; (b) the effects, on both fruits and leaves, of fumigation with different dosages of HCN, at atmospheric pressure and at partial vacuum; and (c) the effects of such fumigation on leaves picked and fumigated at different times of day. Such information is of importance especially in relation to the effects of atmospheric and vacuum fumigation on living tissues.

Materials and methods

The fruits and leaves used in these experiments were those of the Valencia orange, *Citrus Aurantium* L. The fruits were yellow but immature. Only mature leaves were used.

A given number of fruits of average size was taken from different portions of each of 10 trees at each picking. Two samples of 15 fruits each, and 1 of 10 fruits, were then taken at random from the larger collection for each fumigation. Absorbed HCN was recovered from the first 2 samples; the third sample was set aside to determine the relative percentages of injury to the fruits, caused by the different conditions of fumigation.

The fruits for the first series of experiments (table I) were picked between January 20 and February 4, 1942. The equatorial diameters of these fruits ranged from $2\frac{1}{4}$ to $2\frac{3}{8}$ inches, with a mean of $2\frac{5}{16}$ inches. The fruits for the second series (table II) were a little more nearly mature. They were picked between February 25 and March 24, 1942, and their diameters ranged from $2\frac{7}{16}$ to $2\frac{9}{16}$ inches, with a mean of $2\frac{1}{2}$ inches. Fruits were picked in the late afternoon, the samples were then selected and weighed, and the total surface area of each sample was determined. The fruits were then conditioned overnight at 66° to 68° F. before being fumigated. All samples were conditioned at approximately the same temperature because temperature is an important factor in governing the amount of HCN that tissues will absorb (**2**). After removal from the fumatorium, the fruits

were transferred to another laboratory, cut into small pieces, and put into the distillation flasks. This procedure took about 20 minutes.

A little over 300 grams of mature leaves were taken at random from all sides of 8 or 10 trees at each picking. The leaves were thoroughly mixed in the laboratory, and three 100-gram samples were weighed out. Two of these samples were placed in large, flat wire baskets to be fumigated. The third sample of each lot was used for moisture determination. These samples were held at 100° C. for 1 hour and were then dried to constant weight at 65° C. in a mechanically ventilated oven. The leaves were not temperature-conditioned before fumigation, as the fruits had been, but were put into the fumatorium about 15 or 20 minutes after the last leaves of a given set of samples had been removed from the trees.

Because of the subsequent wilting and drying of the leaves, it did not seem feasible to attempt to determine the effects of the different experimental conditions on the relative susceptibility of the leaves to fumigation injury.

After removal from the fumatorium, the leaves were exposed to the air 1 minute before being put into distillation flasks. Previous experiments (1) had shown that this was enough time to permit the escape of the HCN that had been adsorbed on the surface of the leaves. For the first series of tests (table III), leaves were picked at approximately 8:00 A.M. and 1:00 P.M.; for the second series (table IV), they were picked at 8:00 and 10:00 A.M. and at 1:30 and 3:30 P.M.

For fumigations at atmospheric pressure, the liquid HCN was dispensed from a burette into a circulating attachment on the fumatorium, where it was, in turn, caught up by a stream of air and gasified before entering the fumatorium. For the vacuum fumigations it was, of course, unnecessary to resort to mechanical means in order to gasify the liquid HCN. After entering the fumatorium, the gas was kept in circulation by means of a fan which ran during each entire fumigation period. Reduced-pressure fumigations were made at a sustained partial vacuum of approximately 26 inches, and at barometric pressures of 28.5 to 28.9 inches. All fumigations were made in a metal fumatorium having a capacity of 100 cu. ft., with liquid HCN of 96+ per cent. purity, and at a temperature of 75° F. No attempt was made to control the humidity in the fumatorium in these experiments. Fumigations were made at the relative humidities which prevailed at the time the fumigations were made. For the fruit fumigations the relative humidities ranged from 20 to 67 per cent., but for the leaf fumigations they were not determined.

Methods for the recovery of HCN from the fumigated tissues, and for the measurement of the HCN recovered, have been described in a previous paper (1) and are not repeated here. Some further specific details of the methods used in these experiments are given in appropriate sections of the text.

Results

Experiments with Valencia-orange fruits

Effect of different fumigation periods.—The greater the length of time of exposure to the fumigant, the greater the percentage mortality of the insect pest; but the effect of the fumigant on the tissues of the host must also be considered. The object of this series of experiments, therefore, was to determine the effect of the length of time of exposure on the fruits in the fumatorium, as indicated by the relative amounts of HCN recovered and the relative numbers of fruits injured. All fumigations in this series were

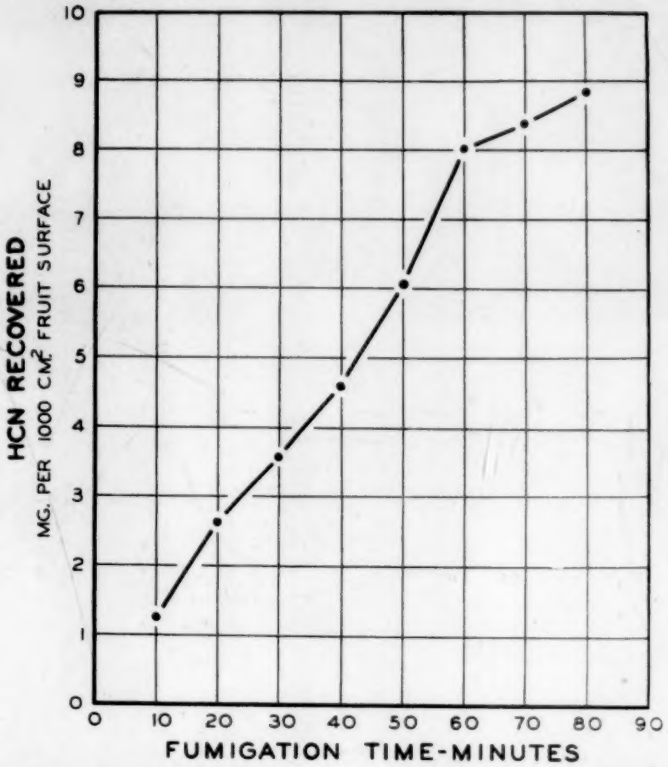

Fig. 1. Relative amounts of HCN recovered from Valencia-orange fruits fumigated at atmospheric pressure, with 8 ml. of HCN, for different lengths of time. (See table I.)

made at atmospheric pressure. The amounts of absorbed HCN recovered from fumigated orange fruits, after different periods of exposure to a given concentration (8 ml.) of HCN, are shown in figure 1 and table I.

The data presented in figure 1 show that the increase in mean amounts of HCN absorbed (as indicated by the amounts recovered) were almost linear for each 10-minute period from 10 to 60 minutes; after that, the curve begins to flatten, and, as shown in table I, the amounts of HCN recovered were more variable. The decrease in recovery values which caused the curve

TABLE I

THE EFFECT OF DIFFERENT EXPOSURE PERIODS UPON THE AMOUNT OF HCN RECOVERED FROM VALENCIA-ORANGE FRUITS FUMIGATED IN A 100-CU. FT. FUMATORIUM, AT ATMOSPHERIC PRESSURE, WITH 8 ML. HCN*

SAMPLE†	TOTAL WEIGHT OF SAMPLE†	TOTAL SURFACE AREA OF SAMPLE	FUMIGATION EXPOSURE PERIOD	HCN RECOVERED PER 1,000 CM.² OF FRUIT SURFACE
	gm.	*cm.²*	*min.*	*mg.*
1	1,646	1,701.5	10	1.3
2	1,636	1,704.2	10	1.2
3	1,650	1,710.9	10	1.3
4	1,658	1,741.6	10	1.3
Mean	.....	.....	.....	1.3
5	1,532	1,624.9	20	2.3
6	1,530	1,616.5	20	2.6
7	1,481	1,584.3	20	3.0
8	1,525	1,603.5	20	2.6
Mean	.....	.....	.....	2.6
9	1,546	1,636.9	30	3.4
10	1,480	1,591.5	30	3.3
11	1,491	1,558.9	30	3.7
12	1,477	1,561.5	30	4.0
Mean	.....	.....	.....	3.6
13	1,468	1,579.5	40	4.2
14	1,450	1,572.3	40	4.8
15	1,459	1,568.7	40	4.7
16	1,456	1,567.5	40	4.7
Mean	.....	.....	.....	4.6
17	1,546	1,617.7	50	5.7
18	1,522	1,604.7	50	6.4
19	1,544	1,652.7	50	6.2
20	1,562	1,632.1	50	5.9
Mean	.....	.....	.....	6.1
21	1,550	1,647.9	60	8.4
22	1,546	1,649.1	60	7.9
23	1,405	1,532.7	60	8.1
24	1,397	1,526.7	60	7.8
Mean	.....	.....	.....	8.1
25	1,410	1,532.7	70	8.4
26	1,406	1,521.9	70	8.3
27	1,647	1,692.3	70	8.7
28	1,642	1,713.5	70	8.3
Mean	.....	.....	.....	8.4
29	1,641	1,681.5	80	9.0
30	1,625	1,659.7	80	8.8
31	1,573	1,662.3	80	8.2
32	1,585	1,668.3	80	9.3
Mean	.....	.....	.....	8.8

* Determinations were made between January 20 and February 4, 1942, at a time when the fruits were beginning to yellow but were still immature.

† Each sample consisted of 15 fruits, all of approximately the same size.

to flatten may have occurred because the fruits had reached the peak of their rate of absorption by the end of the 60-minute period, or because the fruits, having become more heavily charged, had lost a larger percentage of

the HCN before they could be put into the distillation flasks. The maximum capacity of the fruits for the absorption of HCN had not yet been reached, however, for although the curve begins to flatten after the 60-minute period, the trend is still upward for both the 70- and 80-minute exposure periods. Another seemingly possible explanation for the flattening of the curve is that, during the longer fumigation periods (70 and 80 minutes), a larger proportion of the HCN was adsorbed on the walls of the fumatorium and was thus not available for absorption by the fruits. This point will be discussed later in the paper.

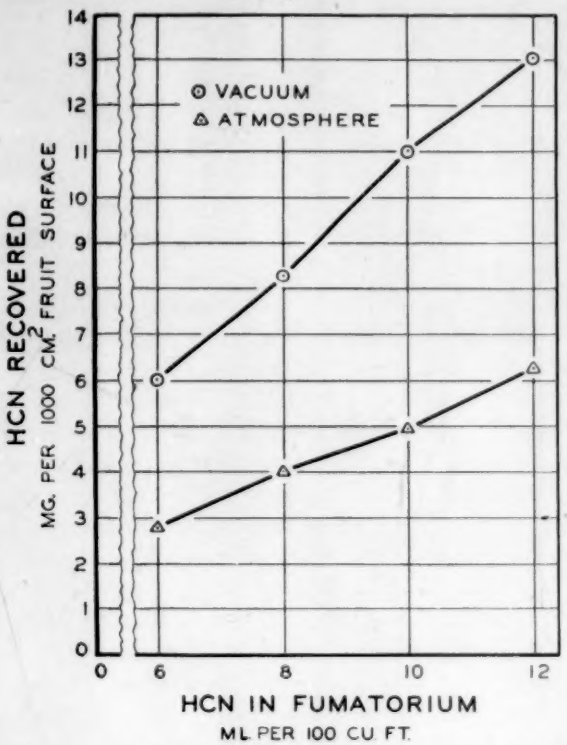

FIG. 2. Relative amounts of HCN recovered from Valencia-orange fruits fumigated at atmospheric pressure and at partial vacuum, with various amounts of HCN. (See table II.)

No fruits were injured at any of the exposure periods up to and including 40 minutes; but at the periods of 50, 60, 70, and 80 minutes 3, 11, 14, and 15 fruits, respectively, were injured. Of these 43 fruits, 35 were slightly, 7 moderately, and 1 (at the 80-minute exposure period) badly injured.

The results of this series of experiments show, therefore, that the length of time of exposure to the fumigant was an important factor in governing, not only the amounts of HCN absorbed, but also the extent of injury to the fruits.

EFFECT OF FUMIGATION AT ATMOSPHERIC AND REDUCED PRESSURES, WITH VARIOUS CONCENTRATIONS OF HCN.—Because vacuum fumigation, as well as

TABLE II

HCN RECOVERED FROM VALENCIA-ORANGE FRUITS FUMIGATED AT ATMOSPHERIC PRESSURE AND AT PARTIAL VACUUM, WITH VARIOUS AMOUNTS OF HCN*

EXPERIMENT†	CONCENTRATION OF HCN IN FUMATORIUM	MILLIGRAMS HCN RECOVERED PER 1,000 CM.² OF FRUIT SURFACE, AFTER FUMIGATION AT	
		ATMOSPHERIC PRESSURE	PARTIAL VACUUM (APPROXIMATELY 26 INCHES)
	ml.	*mg.*	*mg.*
1	6	3.1	4.9
2	6	3.2	5.6
3	6	2.4	6.5
4	6	2.7	7.7
5	6	2.8	6.5
6	6	2.8	5.8
7	6	2.7	5.6
8	6	2.7	5.7
Mean	—	2.8	6.0
9	8	4.3	8.7
10	8	4.2	7.7
11	8	4.0	9.4
12	8	3.7	8.4
13	8	4.0	8.2
14	8	4.0	8.5
15	8	4.0	7.5
16	8	3.9	7.7
Mean	—	4.0	8.3
17	10	4.2	10.8
18	10	4.2	9.3
19	10	5.4	9.7
20	10	5.0	10.3
21	10	4.6	13.3
22	10	5.1	12.3
23	10	6.2	10.2
24	10	5.0	11.9
Mean	—	5.0	11.0
25	12	6.0	11.1
26	12	5.8	11.7
27	12	5.9	13.6
28	12	5.8	10.6
29	12	5.9	11.9
30	12	6.2	13.2
31	12	7.5	17.8
32	12	7.0	14.5
Mean	—	6.3	13.1

* Determinations were made between February 25 and March 24, 1942, at a time when the fruits were yellow but not quite mature. All fumigation exposure periods were 40 minutes long.

† Each experiment included two samples of 15 fruits each. The 64 samples used in these experiments had a mean weight of 1,983 ± 46 grams, and a mean surface area of 1,840 ± 32 sq. cm.

fumigation at atmospheric pressure, is used for killing pests on living plants, it seemed important to determine the relative effects of these two methods

(a) on the amounts of HCN that the plant tissues may absorb during a given fumigation period, and (b) on the extent of injury to the fumigated tissues. The relative amounts of HCN absorbed were determined by measuring the amounts of HCN recovered from fruit samples fumigated at atmospheric pressure, and comparing these with the amounts recovered from similar samples fumigated at a partial vacuum of approximately 26 inches. Four different concentrations of HCN (6, 8, 10, and 12 ml.) were used in the fumatorium in these experiments. The fumigation period was always 40 minutes. The results of these determinations are presented in figure 2 and in table II.

The results in figure 2 show that as the concentrations of HCN in the fumatorium were increased, the amounts of HCN that could be recovered from the samples also increased. The plotted recovery values for both atmospheric and vacuum fumigation form almost straight lines, but values for atmospheric fumigation are a little less than half as great as those for vacuum fumigation, and the graph line for the latter is a little steeper than that for the former.

As shown in table II, the recovery values from paired samples (those of experiments 1 and 2, 3 and 4, and so on) were similar. The 2 samples of a given pair were picked, fumigated, and distilled simultaneously. The different pairs of samples were picked at different times of the day, however, or on different days; this probably accounts for the fluctuation and overlapping of values, both in the groups fumigated at atmospheric pressure and in those fumigated at partial vacuum.

It is probable that for equal concentrations and exposure periods, these samples would have absorbed a little less HCN than those from which the data for table I were obtained. This is indicated by the fact that, as shown in table I for the recoveries at a concentration of 8 ml. HCN and for a fumigation period of 40 minutes at atmospheric pressure, the mean was 4.6 mg. instead of 4.0 mg. per 1,000 sq. cm. of fruit surface. This was to be expected, because the fruits used for the data in table II were tested February 25 to March 24 and were consequently more nearly mature than those tested January 20 to February 4 for the data in table I. Previous experiments (**2**) have shown that while orange fruits are turning from green to yellow or orange in color, they absorb decreasingly smaller amounts of HCN, and that mature fruits absorb much less HCN than young fruits.

The relative numbers of fruits injured by fumigation at atmospheric pressure and at partial vacuum, with the different dosages of HCN, were as follows:

HCN dosage (ml.)	Number of fruits injured		
At atmospheric pressure	Slightly	Moderately	Badly
6	3	0	0
8	6	0	0
10	4	0	0
12	12	0	0

AT PARTIAL VACUUM			
6	7	0	0
8	15	0	0
10	15	2	1
12	13	1	1

Each test included 4 samples of 10 fruits each. The data show that twice as many fruits were slightly injured at partial vacuum as at atmospheric pressure, and that 5 fruits were moderately or badly injured at partial vacuum and none at atmospheric pressure.

These fruits were somewhat more susceptible to injury than less mature fruits. For example, there was no injury to the younger fruits of the preceding experiments (see "Effect of Different Fumigation Periods") when fumigated with 8 ml. of HCN at atmospheric pressure for 40 minutes, while under similar conditions in the present tests, 6 fruits were slightly injured. The data for fruit injury in this experiment also show that some samples of fruit were more susceptible to injury than others. For example, at atmospheric pressure for 40 minutes, 6 fruits were injured with the 8-ml. dosage, and only 4 with the 10-ml. dosage; at partial vacuum 18 fruits were injured with the 10-ml. dosage, and only 15 with the 12-ml. dosage. Such variations have been encountered repeatedly in previous experiments on the effect of HCN on citrus tissues. To a certain extent, the response of the fruit depends more upon its physiological condition than upon the amount of HCN which it absorbs.

The results of this series of experiments show that much less HCN was absorbed and fewer fruits were injured when the fumigations were made at atmospheric pressure than when made at partial vacuum.

EXPERIMENTS WITH VALENCIA-ORANGE LEAVES

HCN RECOVERY FROM LEAVES FUMIGATED AT ATMOSPHERIC PRESSURE AND AT PARTIAL VACUUM, WITH VARIOUS AMOUNTS OF HCN.—The relative effects of fumigations at atmospheric and reduced pressures were determined also on mature Valencia-orange leaves. The reduced pressure used for the leaves was the same as that used for the fruits, that is, a partial vacuum of approximately 26 inches. Five concentrations of HCN (4, 6, 8, 10, and 12 ml.), instead of four, were used in these tests. The exposure periods were always 40 minutes. Two leaf samples were fumigated simultaneously in the morning at atmospheric pressure, and 2 similar samples were fumigated in the early afternoon at partial vacuum. The next day the order was reversed, the partial-vacuum tests being made in the morning and the atmospheric-pressure tests in the afternoon, and so on during the entire series. The results are presented in figure 3 and table III.

Figure 3, in which the points represent the means of recoveries from 2 samples of leaves fumigated simultaneously, shows that there was a gradual increase in the amounts of HCN recovered from the samples as the concentrations of HCN in the fumatorium were increased. The values for any 2

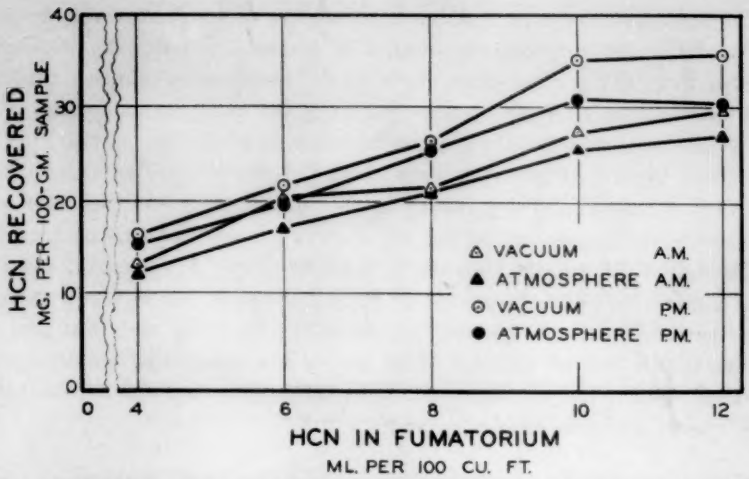

FIG. 3. Relative amounts of HCN recovered from samples of mature Valencia-orange leaves fumigated in the morning and in the afternoon, at atmospheric pressure and at partial vacuum, with various amounts of HCN. (See table III.)

samples fumigated simultaneously (experiments 1 and 2, 3 and 4, and so on) were similar, as shown in table III. There were some fluctuations at the different concentrations of HCN, however, as shown in figure 3, so that while the general trend of the graph lines is upward, they are not entirely linear. Such fluctuations as these are unpredictable and not preventable, at least when one is working with material grown under field conditions.

TABLE III

HCN RECOVERED FROM MATURE VALENCIA-ORANGE LEAVES FUMIGATED IN THE MORNING AND IN THE AFTERNOON, AT ATMOSPHERIC PRESSURE AND AT PARTIAL VACUUM, WITH VARIOUS AMOUNTS OF HCN*

EXPERIMENT	CONCENTRATION OF HCN IN FUMATORIUM	MILLIGRAMS HCN RECOVERED FROM 100-GRAM SAMPLES OF LEAVES FUMIGATED AT			
		ATMOSPHERIC PRESSURE		PARTIAL VACUUM (APPROXIMATELY 26 INCHES)	
		8:00 A.M.	1:00 P.M.	8:00 A.M.	1:00 P.M.
	ml.	*mg.*	*mg.*	*mg.*	*mg.*
1	4	12.3	15.4	13.0	16.4
2	4	12.2	15.8	13.3	16.2
3	6	17.0	19.3	20.0	22.1
4	6	17.5	20.0	20.2	22.9
5	6	16.8	.....	.....	.....
6	6	17.6	.....	.....	.....
7	8	21.8	26.0	22.8	26.6
8	8	20.3	25.3	23.6	26.4
9	10	25.3	30.0	27.5	34.6
10	10	26.5	32.1	27.5	35.3
11	12	27.1	30.3	28.5	35.3
12	12	27.5	30.2	28.9	35.9

* All fumigation periods were 40 minutes long.

The findings of special interest in this series of tests, however, were that (a) the HCN recoveries from samples of leaves fumigated at atmospheric pressure were always less than those from comparable samples fumigated at partial vacuum; (b) the recovery values for both atmospheric pressure and partial vacuum were always less from leaves picked in the morning than from those picked in the afternoon; and (c) the recoveries from samples fumigated at atmospheric pressure in the afternoon were, with one exception (see 6-ml. concentration, fig. 3), always greater than those from samples fumigated at partial vacuum in the morning. Apparently, some condition conducive to the absorption of HCN existed in the leaves in the afternoon—a condition that was not present in the morning and that was more effective than a partial vacuum of 26 inches in causing the leaves to absorb excess amounts of HCN. In order to obtain further evidence on this matter, the following series of tests was made.

HCN RECOVERY FROM LEAVES PICKED AND FUMIGATED AT DIFFERENT TIMES OF DAY.—The fumigations in this series were made at atmospheric pressure only. Each fumigation lasted 40 minutes, and the concentrations of HCN were always 8 ml. The results of these tests are shown in figure 4 and table IV.

The points on the graph line in figure 4, which represent the means of each of the four groups of eight determinations (table IV), show that from 8:00 A.M. to 1:30 P.M. there was an increase in the amounts of HCN that could be recovered from the 100-gram samples of fumigated leaves. With the 3:30 P.M. samples, there was a slight drop in the curve, but the difference between the means at 1:30 and at 3:30 P.M. is barely significant at the 5 per cent. level and not at all at the 1 per cent. level, and is therefore not a valid difference. On the other hand, the differences between the means of the values for the 8:00 A.M. samples and for those of the other three groups of samples are greater than is required for significance at the 1 per cent. level, as determined by the "t" test. This is true, also, of the differences between the means of the values for any two of the first three groups of determinations.

The data in table IV also show that there were differences in the amounts of HCN that could be recovered from leaves picked and fumigated at a given hour, but on different days. For example, for the 8 samples picked at 8:00 A.M. the recovery values ranged from 17.5 to 23.0 mg. The differences, however, between the values for the 2 samples of any of the 16 pairs of samples were usually very small; in fact, in four instances the values for the 2 paired samples were identical (see experiments 1 and 2, and 5 and 6 in the 8:00 A.M. column; experiments 1 and 2 in the 1:30 P.M. column; and experiments 7 and 8 in the 3:30 P.M. column of table IV).

As shown in table IV, there was some overlapping of the recovery values in the four groups of determinations. For example, the values for the 1:30 P.M. determinations were not always higher than those for the 10:00

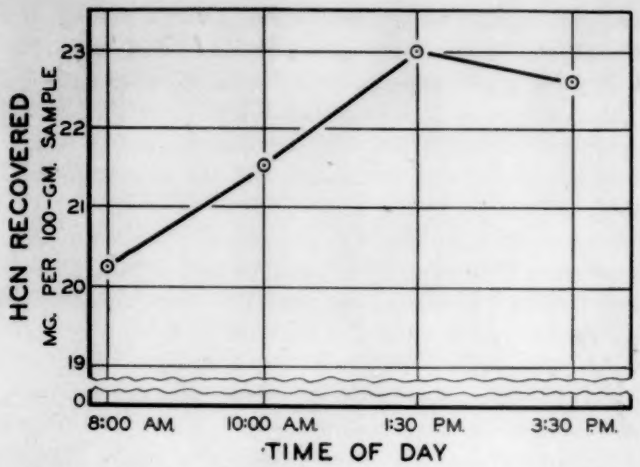

FIG. 4. Relative amounts of HCN recovered from Valencia-orange leaves picked and fumigated at intervals during the day. Each point on the curve represents the mean of 8 determinations. All fumigations were made at atmospheric pressure, with 8 ml. of HCN. (See table IV.)

A.M. determinations; the same is true for the values determined at 10:00 A.M. and at 8:00 A.M. These variations were probably due to the fact that the samples were picked on different days. It is unfortunate that conditions would not permit the making of a larger number of tests at each of the given times of day. It is also unfortunate that leaves could not have been picked and fumigated later in the day, say at 5:00 or 6:00 P.M. Could this have been done, it is probable that the recovery values for that time of day

TABLE IV

HCN RECOVERY FROM MATURE VALENCIA-ORANGE LEAVES PICKED AND FUMIGATED AT VARIOUS TIMES OF DAY*

EXPERIMENT	PERCENTAGE OF MOISTURE IN LEAF SAMPLES				HCN RECOVERED (MILLIGRAMS PER 100-GRAM SAMPLE)			
	8:00 A.M.	10:00 A.M.	1:30 P.M.	3:30 P.M.	8:00 A.M.	10:00 A.M.	1:30 P.M.	3:30 P.M.
	%	%	%	%	*mg.*	*mg.*	*mg.*	*mg.*
1	58	58	57	57	19.8	22.0	24.3	22.8
2	58	58	57	57	19.8	23.2	24.3	23.0
3	58	58	57	56	23.0	22.8	23.5	23.7
4	58	58	57	56	22.3	23.2	23.9	24.5
5	56	56	55	54	17.5	20.1	22.1	21.0
6	56	56	55	54	17.5	20.3	22.0	21.4
7	55	56	54	53	21.0	20.3	21.9	22.3
8	55	56	54	53	21.3	20.4	22.1	22.3
Mean†	.....	.....	.....	.....	20.3	21.5	23.0	22.6

* Leaf samples were picked between November 3 and 11, 1943, and were weighed and fumigated immediately after picking. HCN dosages were 8 ml., and each fumigation period was 40 minutes long.

† The differences between the mean of the 8:00 A.M. samples and the means of the other three groups of samples are greater than is required for significance at the 1 per cent level, as determined by the "t" test.

would have been considerably smaller than those obtained at 3:30 P.M., though probably not so small as those obtained at 8:00 A.M. Nevertheless, the results of these experiments, together with those described in the immediately preceding section, indicate that, all other conditions being equal, there is a positive correlation between the time of day at which Valencia-orange leaves are picked and fumigated and the amounts of HCN which they will absorb.

As shown by the data in table IV, the moisture content of the leaves did not appear to be an important factor in determining how much HCN they would absorb during the fumigation period. These results confirm those published by the authors at an earlier date (**2**).

Discussion

There was more or less variation in the amounts of HCN recovered from the different groups of Valencia-orange fruits and leaves fumigated at atmospheric pressure and at partial vacuum in the present experiments (tables I to IV). These variations were no greater than one should expect, however, when working, as in this case, with biological material which in itself is subject to rather rapid changes, and which also responds readily to changes in soil and weather conditions. That sampling methods, fumigation manipulations, or HCN recoveries and measurements could not have been responsible for the variations in values, is shown by the fact that the amounts of HCN recovered from paired samples of either fruits or leaves were nearly the same; in fact, for some of the pairs the amounts were identical.

The fruits fumigated at atmospheric pressure absorbed a little less than half as much HCN as those fumigated at partial vacuum (fig. 2, table II). The differences for the leaves (fig. 3, table III) were much smaller: leaves fumigated at atmospheric pressure at 8:00 A.M. absorbed, on the average, only 10 per cent. less HCN than those fumigated at partial vacuum at 8:00 A.M.; for those fumigated at 1:00 P.M., the difference between the means at the two pressures was only slightly greater—11 per cent. The differences between the relative amounts of HCN recovered from leaves and from fruits, when fumigated at atmospheric pressure or at partial vacuum, were probably influenced to a certain extent by the rates of penetration of the HCN into the tissues. On the other hand, however, it seems reasonable to assume that the HCN saturation point would be reached sooner in the thin leaves than in the more massive fruits.

Aside from the effects of anatomical and physiological differences in the tissues of leaves and fruits, there is also the possibility that the rather large differences in the percentages of moisture may have been a factor in HCN recovery. The moisture content of the leaves fumigated at the different pressures, although not shown in table III, was 56 to 58 per cent., which was, on the average, just a little higher than the moisture contents shown in table IV for the leaves fumigated at intervals during the day. The water

content of the fruit samples was not determined, but judged by previous results, the peel of these fruits contained approximately 70 per cent. moisture, and the remainder of the fruit approximately 85 per cent. Small differences in the moisture content of tissues do not appear to affect their HCN-absorbing capacity, but these differences were fairly large and may have been a factor.

Differences in relative humidities during fumigation periods did not appear to have any effect on the amounts of HCN recovered from the fruits. For this reason no records were kept of the relative humidities during fumigation of leaf samples. To summarize the results of the limited amount of work that has been done previously on the relation of relative humidity to the absorption of HCN by living plant or animal tissues, it may be said that preexisting relative humidities may have an indirect effect in that they may cause influencing changes in the tissues; but that the relative humidities which exist during the fumigation period appear to have little or no effect unless they are extremes, below 40 or above 85 per cent.

The determination of the possible relation of stomata to the absorption of HCN by citrus fruits and leaves was not one of the objects of these experiments, but the following discussion in this connection seems apropos. In a previous article (**2**), the authors stated that the stomata did not appear to be important in regulating the absorption of HCN. This conclusion was based on the results of fumigations made in the field, in porous tents. In those experiments it was found that the amounts of HCN recovered from fruits and leaves taken from trees fumigated during the day, when the stomata were open, were no greater than those from trees fumigated in a similar manner at night, when the stomata were closed. Under the conditions of those experiments, only about 5 per cent. of the original amount of HCN remained in the tents at the close of the 45-minute exposure period. With such a decrease in the amount of HCN in the tent, it is very likely that during the latter part of the fumigation period a portion of the absorbed HCN escaped from the fruits and leaves even before the tent was removed from the tree. On the other hand, because of the relatively rapid loss of HCN from the tents, the fruits and leaves absorbed less than they would have absorbed had the concentration of HCN remained higher. The important point in this connection, however, is the fact that the concentrations of HCN in the tents were as high for the fumigations made in the daytime as for those made at night [see table I (**2**)]. Had the stomata been the controlling factor, therefore, it should have been possible to recover larger amounts of HCN from the fruits and leaves fumigated in the daytime than from those fumigated at night; this was not the case.

In the earlier studies (**2**), the authors also found that, with the loads of fruits or leaves used in a fumatorium in laboratory experiments (loads similar in size to those used in the present laboratory experiments), and with 8 ml. of HCN, the concentration of HCN in the fumatorium remained remarkably constant during the 40-minute fumigation period. The means of

156 determinations showed that, with an 8-ml. dosage, there were 1.45 mg. of HCN per liter of space in the fumatorium at the end of the 1-minute period, and 1.33 mg. per liter at the end of the 40-minute period—a decrease of only 8.3 per cent. These results indicate that the decreased amount of HCN in the fumatorium at the end of the fumigation period was largely due to its absorption by the tissues, and not to its adsorption on the walls of the fumatorium. Lindgren[3] made further extensive tests on this fumatorium when empty and when loaded, or partially loaded, with different commodities. He found, for example, that, with a 10-ml. dosage but no commodity, the fumatorium contained only 21.7 per cent. less HCN after 6 hours than after 1 minute. Therefore, it was to be expected that, under otherwise comparable conditions, more HCN could be recovered from fruits and leaves that had been fumigated in a fumatorium in the laboratory than from those fumigated in porous tents in the field, and that any difference between the amounts of HCN absorbed at any given time of day could be more accurately determined by the former method of fumigation than by the latter.

The fact that, in the present experiments, less HCN was recovered from the samples of leaves picked and fumigated at 8:00 A.M. than from those picked and fumigated later in the day (tables III, IV) might be interpreted as indicating that the stomata may have been an important factor in governing the amount of HCN absorbed by the leaves during the fumigation period. Such a conclusion would be in agreement with the findings of Clayton (**3**), but would be contrary to the results of STONE (**11**) and MOORE (**7**), who concluded that other attributes of the tissues are more important than the stomata in determining the amounts of HCN which leaves will absorb when fumigated. It is well known, for example, that plant tissues become more permeable upon being exposed to sunlight. The direct rays of the sun, for that day, had not fallen on the leaves that were picked at 8:00 A.M. Furthermore, metabolic changes in the leaf tissues may have been more important than the stomata in governing the absorption of HCN by the leaves. This is indirectly indicated by the results of previous experiments (**2**), which have shown that while orange fruits are turning from green to yellow or orange in color, they absorb decreasingly smaller amounts of HCN. It does not seem probable that a commensurate change in stomatal activity could have occurred within such a short time. The possible function of the stomata in governing the absorption of HCN by fumigated citrus fruits and leaves cannot be considered to be definitely answered until further evidence has been presented.

Summary

The experiments reported in this paper deal particularly with (a) the effect, on Valencia-orange fruits, of exposure to HCN for different lengths of time, at atmospheric pressure; (b) the effects, on both fruits and leaves, of fumigation with different dosages of HCN, at atmospheric pressure and at partial vacuum; and (c) the effects of such fumigation on leaves picked

[3] LINDGREN, D. L. Unpublished data. 1942.

and fumigated at different times of day. Visible injury to fruits, but not to leaves, was noted and recorded.

As determined by recovery values, the amounts of HCN absorbed by Valencia-orange fruits increased almost linearly for each 10-minute exposure period from 10 to 60 minutes; after that, the curve began to flatten.

With dosages of 6, 8, 10, and 12 ml. HCN per 100 cu. ft., fruits fumigated in a fumatorium at atmospheric pressure absorbed a little less than half as much HCN as fruits fumigated at a partial vacuum of 26 inches.

Approximately the same amounts of HCN were absorbed by fruits exposed to an 8-ml. dosage for 70 or 80 minutes at atmospheric pressure as by fruits exposed to a similar dosage for 40 minutes at partial vacuum.

In experiments where the concentrations of HCN were constant but where the lengths of exposure periods varied, no fruits were visibly injured at any of the 10-minute intervals below 50 minutes, but at the longer periods of exposure (50, 60, 70, and 80 minutes) the numbers of fruits injured became increasingly greater. In the experiments where the length of exposure period was constant but the concentrations of HCN varied, about half as many fruits were injured at atmospheric pressure as at partial vacuum.

The results of the experiments with Valencia-orange leaves showed that (a) the amounts of HCN absorbed by leaves fumigated at atmospheric pressure were always less than the amounts absorbed by leaves fumigated at the same time of day but at partial vacuum; (b) the amounts of HCN absorbed by leaves fumigated either at atmospheric pressure or at partial vacuum were always less in leaves picked and fumigated in the morning than in those picked and similarly fumigated in the afternoon; (c) the amounts of HCN absorbed by leaves fumigated at atmospheric pressure in the afternoon were, with one exception, greater than the amounts absorbed by leaves fumigated at partial vacuum in the morning; (d) leaves picked at 8:00 A.M. absorbed less HCN than those picked at 10:00 A.M., 1:30 P.M., or 3:30 P.M.; and (e) the amounts of HCN absorbed did not appear to be influenced by such variations as occurred in the moisture content of the different samples of leaves.

The possible function of the stomata in governing the absorption of HCN by citrus fruits and leaves is discussed.

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ASHING PROCEDURES FOR THE DETERMINATION OF COPPER IN PLANT MATERIAL[1]

L. F. Bailey and J. S. McHargue

In some recent plant analyses difficulty was encountered in obtaining consistent results for copper. The cause of this inconsistency was found to be associated with the method used for ashing the plant material. This paper contains the results of an experiment to ascertain the effect of different ashing procedures for the determination of copper in tomato and alfalfa plant materials.

Ashing procedure

Dry ashing

Two-gram samples were ignited in a muffle furnace at 450° and 650° C. for different periods of time. Porcelain, platinum, and silica dishes were used for ashing. The ash was dissolved in 15 ml. of 1 : 1 hydrochloric acid and evaporated to about 5 ml. on an electric hot plate.

Wet ashing

Three mixtures were used for wet digestions: nitric and sulphuric acids, 30 per cent. hydrogen peroxide and sulphuric acid, and selenium and sulphuric acid.

In the nitric acid digestion 3 ml. of concentrated sulphuric acid was used for each gram of dry plant material, a total of 50 ml. of concentrated nitric acid was added in four portions, the mixture being evaporated to white fumes and cooled between successive additions. A description of this well-known procedure is given by Piper (**7**).

Lindner and Harley (**5**) recently described a procedure using 30 per cent. hydrogen peroxide. The digestion is rapid and easily manipulated. Sulphuric acid is used with successive additions of small amounts of the peroxide. A mixture of three ml. of concentrated sulphuric acid with one gram of dry plant material is heated to partially decompose the organic matter and the digest is then cooled. In the present study three ml. of peroxide was added and a small flame used for digestion to white fumes after which the mixture was cooled. This was repeated with one ml. of hydrogen peroxide and with four to six additions of five drops of the peroxide.

Selenium is frequently used to catalyze the oxidation of organic matter with sulphuric acid prior to nitrogen determinations. This method is also suitable when copper is to be determined. Six ml. of concentrated sulphuric acid was used for a gram of plant material. To this was added one gram of potassium sulphate and 0.5 gram of powdered selenium. The selenium need

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

not be weighed accurately. The solution clears in about 30 minutes, and no further digestion is necessary for copper determination.

In each of these wet digestions the sulphuric acid must be neutralized prior to analysis for copper. This requires a rather large amount of alkali which may carry an appreciable quantity of copper. A neutralized blank more than compensates for this added copper because the blank always requires more hydroxide for neutralization than the plant solution. The reagent-copper subtracted from the total copper obtained gives too low a value for the plant material. This error can be avoided by using copper-free (double-distilled) ammonium hydroxide for neutralization. This procedure has given consistently reliable results.

Determination of copper

Several methods are available for the determination of small amounts of copper in plant materials (**9**). One of the most satisfactory is the colorimetric procedure based on the use of sodium diethyldithiocarbamate which forms the strongly-colored copper carbamate. HADDOCK and EVERS (**4**) have discussed the early development of this analytical procedure. More recently, SHERMAN and MCHARGUE (**8**) described a modified carbamate procedure which they used for soils. Conditions necessary for the control of certain interfering ions are adequately described in the literature (**1, 2, 7**) and need not be repeated here.

Several hundred determinations have been made in the laboratory using a modification of the SHERMAN and MCHARGUE (**8**) procedure. The acid solutions of the digested samples are passed through acid-washed filter paper and neutralized with copper-free ammonium hydroxide, using methyl red as an indicator. Two ml. of N hydrochloric acid are added, the solution transferred to a separatory funnel, and double-distilled water added to make a volume of 50 ml., as indicated by a rough graduation on the separatory funnel. This volume need not be exact. If more than 0.03 mg. of copper are present in the sample, the acid solution of the plant ash should be made to 50 ml. in a volumetric flask and a suitable portion of this taken for neutralization and subsequent analysis.

To the solution in the separatory funnel add 25 ml. of citrate buffer (**8**), 0.5 ml. of concentrated ammonium hydroxide, and 10 ml. of 0.1 per cent. sodium diethyldithiocarbamate in water. Extract the copper carbamate formed with four successive portions of 2.5 ml. of carbon tetrachloride. Colorimetric comparisons can be made with a photoelectric colorimeter and a blue filter with maximum transmission at 410 mμ. The preparation of the standard curve for copper is described by SHERMAN and MCHARGUE (**8**).

Sodium diethyldithiocarbamate in water solution is quite stable. A solution kept in the laboratory for 60 days showed no tendency to deteriorate. With special precautions it may be kept much longer (**7**). The copper carbamate complex in carbon tetrachloride is stable for at least 24 hours and probably considerably longer. The color intensity of the copper complex is

independent of the amount of ammonium hydroxide used for neutralization. Methyl red indicator is yellow in aqueous solutions but this color is not transferred to the carbon tetrachloride. A mixed indicator of methyl red and methylene blue has a more pronounced end-point but the methylene blue imparts an interfering color to the carbon tetrachloride.

Experiments with dry ashing

Porcelain crucibles

New porcelain crucibles gave dependable values for copper for some time. After repeated use results obtained with them were erratic and always higher than expected. When the ashing temperature was increased from 450° to 650° C. the amount of copper recovered increased. Drastic cleaning procedures augmented this contamination. Copper from inorganic solutions evaporated to dryness in the crucibles could be recovered without error. Higher temperatures, such as those required for ashing, were involved in this source of error. These results indicate that porcelain containers are unsatisfactory for ashing plant materials when copper is to be determined, at least if the surface glaze has deteriorated somewhat.

Platinum crucibles

The use of platinum for ashing gave surprisingly high results for copper. Tomato dry matter containing 0.0052 mg. of copper gave values three or more times too high when ashed in platinum (table I). This error was particularly pronounced when ashing at 650° C. for 15 hours. At this temperature for one hour, or at 450° C. for two hours, contamination was slight (table I). As with porcelain, inorganic solutions of copper evaporated to dryness, but not subjected to ashing temperatures, could be recovered accurately. These results suggested that platinum might be interfering in the color reaction by forming a colored carbamate complex.

To test this possibility platinum black was thoroughly extracted with hot hydrochloric acid to remove any copper present. It was then dissolved in aqua regia and evaporated to a hydrochloric acid solution of platinic chloride. This solution when analyzed with the regular carbamate procedure gave a pronounced yellow color reaction almost identical with that shown by copper. The color was proportional to the concentration of the platinic chloride. No reference has been found in the literature to this carbamate reaction of platinum. Platinum interference appears to be inherent in the carbamate procedure but probably would not influence other methods for determining copper.

It remains to be explained how small amounts of platinum are removed from the crucibles during ashing. Higher oxides of manganese are formed during ashing which are reduced by hydrochloric acid with a release of chlorine. Platinic chloride could be formed in this manner. However, the use of various reducing agents prior to dissolving the ash in hydrochloric

acid, or dissolving the ash in sulphuric acid, failed to prevent the platinum effect. Using sulphuric acid to dissolve the ash also eliminates the possible formation of small amounts of aqua regia from any residual nitrates and hydrochloric acid. No satisfactory explanation can be given for the manner in which platinum becomes associated with the ash.

TABLE I

DRY ASHING: THE EFFECT OF CONTAINER, TEMPERATURE, AND LENGTH OF ASHING PERIOD ON THE DETERMINATION OF COPPER IN TOMATO AND ALFALFA DRY MATTER

PLANT MATTER	ASHING CONTAINER	ASHING TEMPERATURE	ASHING TIME	COPPER PRESENT*	COPPER FOUND
		0° C.	*hrs.*	*mg.*	*mg.*
Tomato	Platinum	650	15	0.0052	0.0153 0.0183 0.0158 0.0179
Tomato	Platinum	650	1	0.0052	0.0066 0.0074 0.0054
Tomato	Platinum	450	2	0.0052	0.0055 0.0076 0.0065
Tomato	Silica	450	15	0.0062	0.0049 0.0062 0.0062
Tomato	Silica	650	2	0.0062	0.0060 0.0062 0.0063
Tomato	Silica	650	2	0.0212†	0.0180 0.0161 0.0175
Alfalfa	Silica	450	15	0.0111	0.0111 0.0114 0.0107
Alfalfa	Silica	650	2	0.0111	0.0097 0.0103 0.0096

* As determined by wet ashing.
† 0.015 mg. Cu added as $CuSO_4$.

SILICA DISHES

Plant material ashed in silica dishes shows a loss of copper, the extent of this loss depending on the temperature and time used for the ashing (table I). At 450° C. for 15 hours results are fairly satisfactory, but a certain amount of irregularity occurs. Ashing at 650° C. for two hours permitted a good recovery of copper with tomato plant material but not with alfalfa (table I). When copper sulphate was added to the plant material, some of this added copper was not present in the final determination.

An inorganic source of copper ($CuSO_4$) subjected to ashing temperatures in silica dishes cannot be completely recovered. The amount of the re-

covered copper decreases with increasing temperature and time. In one test 93 per cent. of the added copper was not regained (650° C. for 15 hours).

It is unlikely that this poor recovery of copper can be explained as a loss through volatilization, since copper compounds are relatively heat stable. PIPER (7) offers a more plausible explanation. High temperatures favor the formation of complex metal silicates which are not soluble in hydrochloric acid. Trace elements, particularly, form these complexes. The silica present in plant material and the silica dishes used in ashing probably fixed the copper in forms not soluble in hydrochloric acid. GREENLEAF (3) noted

TABLE II

WET ASHING: A COMPARISON OF THE EFFECT OF DIFFERENT METHODS ON THE DETERMINATION OF COPPER IN TOMATO AND ALFALFA DRY MATTER

PLANT MATTER	DIGESTION MIXTURE	COPPER FOUND	AVERAGE COPPER FOUND
		mg.	*mg.*
Tomato	$HNO_3 + H_2SO_4$	0.0052	
		0.0052	
		0.0056	
		0.0050	0.0052
Tomato	$H_2O_2 + H_2SO_4$	0.0047	
		0.0053	
		0.0049	
		0.0051	0.0050
Tomato	$Se + H_2SO_4$	0.0051	
		0.0047	
		0.0055	0.0051
Alfalfa	$HNO_3 + H_2SO_4$	0.0109	
		0.0110	
		0.0114	0.0111
Alfalfa	$H_2O_2 + H_2SO_4$	0.0108	
		0.0107	
		0.0112	0.0109
Alfalfa	$Se + H_2SO_4$	0.0114	
		0.0113	
		0.0109	0.0112

a loss of copper in dry ashing which he suggested might be due to fixation on the ashing receptacles. The type of container used by GREENLEAF was not stated. Silica complexes are not formed when wet digestion procedures are used (7).

Experiments with wet ashing

The three wet-digestion procedures previously described were compared using tomato and alfalfa materials. The data presented in table II are typical of numerous tests that were made. The three methods are in good agreement. On the basis of speed and ease of manipulation the hydrogen peroxide digestion seems to be the most promising. LINDNER (6) recently applied this method to the preparation of plant materials for analysis of several macronutrients. The results for copper given here suggest the suit-

ability of this technique for trace elements in general. The selenium digestion is least desirable because of the large amount of sulphuric acid that must be neutralized. The digestion is also somewhat more difficult to accomplish by this method. PIPER (7) prefers a digestion utilizing perchloric acid. This procedure was not included in the present study.

Discussion

It is doubtful if dry ashing can be considered reliable when copper is to be determined in plant materials. Porcelain and silica containers alter the copper content of the recovered ash. Platinum may be satisfactory when sodium diethyldithiocarbamate is not used to detect the copper.

It is possible that a suitable dry ashing procedure might be established for any specific plant matter but the exact conditions would have to be carefully determined. Reasonably reliable values for copper in alfalfa material can be obtained by ashing at 450° C. for 15 hours in silica dishes. Results for tomato plants ashed in silica dishes at 650° C. for no longer than two hours are fairly satisfactory.

Wet ashing procedures are more rapid and are much less subject to contamination from outside sources of copper. This advantage is a very important one. The three methods described here are all satisfactory but the hydrogen peroxide digestion of LINDNER (5, 6) is apparently the most promising.

Summary

1. Porcelain and silica dishes used for ashing tomato and alfalfa plant materials altered the copper content of these materials. Platinum crucibles interfered in the determination of copper with sodium diethyldithiocarbamate.

2. Wet ashing appears to be much more reliable when copper is to be determined. Results from three wet ashing procedures were in good agreement.

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PHOTOSYNTHESIS IN MAIZE AS INFLUENCED BY A TRANSPIRATION-REDUCING SPRAY[1]

C. G. Barr

(WITH FOUR FIGURES)

Introduction

It has been found that wax and oil emulsions developed for the prevention of desiccation of shrubs and trees in transplanting can be used also, under certain conditions, to reduce the amount of water lost by transpiration in field-grown plants. A waxy coating on the leaves which reduces water loss, however, may interfere with the exchange of carbon dioxide and oxygen between the leaf and its environment. An ideal material to be used primarily for the reduction of transpiration should possess the characteristics of being highly permeable to carbon dioxide and oxygen. No material has been found, however, which has these attributes.

Although the unfavorable effects of waxy coatings on the photosynthetic process are recognized, the net effect has been largely a matter of conjecture or based on casual observations of yield and general appearance of the plants so treated.

An extensive program was carried out during the summer of 1943 to determine the effect on the yield and performance of a number of species of plants by the application of an emulsion that has been developed at this station for the purpose of reducing transpiration. A more detailed report of the entire investigation is to be published later. The present paper is concerned with the results of some specific physiological studies incident to the investigations on the use of certain wax and oil emulsions as sprays.

Reasonable understanding of the photosynthetic process will be assumed in order to save unnecessary repetition. The classic works of Stiles (**12**) and Spoehr (**11**) are familiar to all students of plant physiology. A comprehensive review of the literature on the subject up to 1936 has been given by Miller (**9**). Excellent reviews of the more recent advances in the study of photosynthesis have been made by Emerson (**5**), Gaffron (**6**), and Johnston and Meyers (**7**).

The diurnal variations of the carbohydrates in the leaves of corn are clear cut, reaching a maximum value sometime between 2 and 6 P.M. (**1, 8**). In general the conditions of sunlight and temperature are favorable for maximum photosynthesis during that part of the day. The corn plant, therefore, is quite suitable for a study of carbohydrate formation in the green leaf as affected by the application of a spray material.

Experimental methods

Zea mays, horticultural variety Golden Cross Bantam, was used for the investigation reported herein. The emulsion used was one referred to in a

[1] Contribution from the Michigan Agricultural Experiment Station. Published with the permission of the Director as Journal Article no. 711 (n.s.).

previous paper (**3**), the basic constituents of which consist of a mixture of paraffine and vegetable oils. The exact composition of the spray is considered to be incidental to the problem at hand and the details for its composition will be presented in a forthcoming publication. On the afternoon of August 9, 1943, when the corn was in the early ear-shoot stage of development, a plot consisting of about 200 hills was sprayed with a C/8.5 dilution (1 part of the stock emulsion + 7.5 parts of water, by weight, of a stock emulsion). Beginning at 7 A.M. (Eastern War Time) the following day, August 10, a series of leaf samples was collected at three-hour intervals for a period of 24 hours from sprayed and control plants. The control plants were in an adjoining plot, of the same age, and similar in all respects. Another series of samples was collected from these same plots 20 days later on August 30.

The first collection was made reasonably soon after the application of the spray to minimize any harmful effects of the emulsion on the chlorophyll content and on protoplasmic conditions which might in turn be manifest in the photosynthetic activity.

At the time of the second collection the corn was in the early roasting-ear stage. This collection was taken for the purpose of studying the durability of the emulsion and its after-effect on the permeability to gases, particularly to carbon dioxide.

Samples and analyses

Samples were obtained from a composite of stalks picked at random from a 100-foot row at each sampling. The stalks with their leaves were cut, taken immediately to the laboratory, and two alternate leaves were stripped from the central portion of each of 10 stalks. These were split down the midrib and duplicate 50-gm. samples of the leaf blade were weighed on a torsion balance and rapidly killed in boiling 95 per cent. alcohol. Duplicate leaf-punch samples consisting of 100 punches each were taken from the other half of the leaf used for the alcohol samples. The samples for the sugar analyses were later transferred to 123 × 43 mm. thimbles of Soxlet extractors and extracted to completion with ethyl alcohol. The extracting liquor was made to volume and aliquot portions were used for sugar determinations. The alcohol was removed by evaporation and the resulting water solution cleared with neutral lead acetate. Reducing sugars and total sugars (after invertase hydrolysis) were determined by the Munson-Walker (**10**) method for the precipitation of the cuprous oxide. A modification of the Bertrand (**2**) method was used for the estimation of copper.

Leaf-punch samples were dried to constant weight at 65° C. under vacuum. The dry material was then transferred to 200-ml. Erlenmeyer flasks containing 100 ml. of 1 plus 20 HCl (1 part concentrated hydrochloric acid plus 20 parts water by volume) and hydrolyzed at 120° C. for 1 hour. The hydrolysate was neutralized and made to volume and determinations were made for the glucose equivalent on 50-ml. aliquots by the methods described above for sugars. Total carbohydrates were estimated as glucose and the results expressed as mg. of glucose per 100 gm. of fresh tissue.

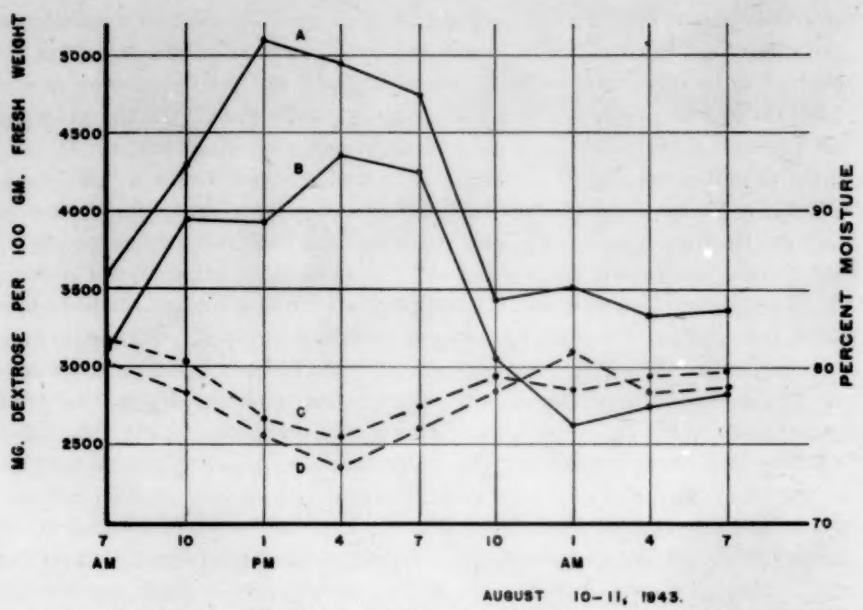

FIG. 1. Variation in the total carbohydrates and the moisture content of maize leaves; August 10–11, 1943. (A) Total carbohydrates, control. (B) Total carbohydrates, sprayed. (C) Moisture content, control, in percentage. (D) Moisture content, sprayed, in percentage.

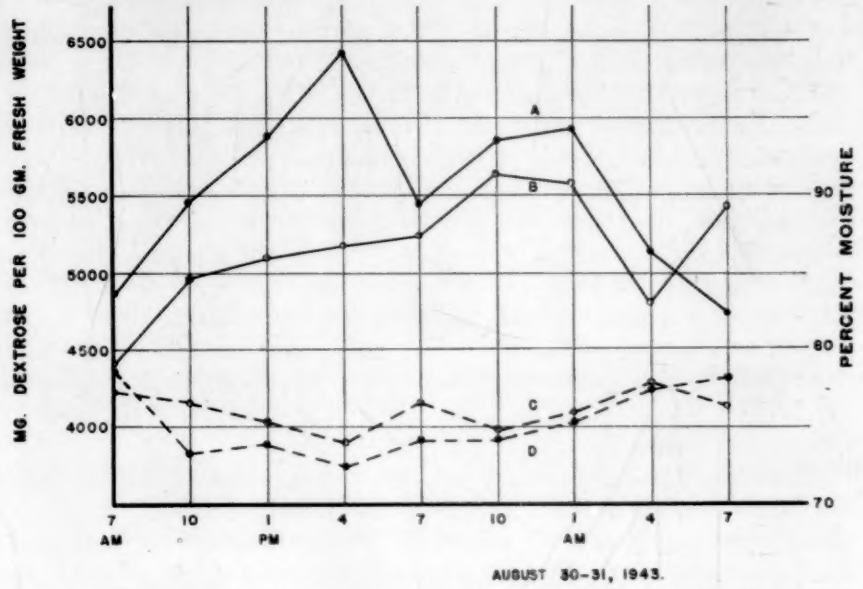

FIG. 2. Variation in the total carbohydrates and the moisture content of maize leaves. August 30–31, 1943. (A) Total carbohydrates, control. (B) Total carbohydrates, sprayed. (C) Percentage moisture content, sprayed. (D) Percentage moisture content, control.

Results and discussion

The results of the determinations, expressed in terms of both fresh and dry weight, are given in tables I, II, and III. Comparable results were obtained by both methods of calculation but the graphs show only the fresh weight data.

TABLE I

MOISTURE AND TOTAL CARBOHYDRATE* CONTENT OF MAIZE LEAVES; LEAF-PUNCH SAMPLES

TIME	MOISTURE	TOTAL CARBOHYDRATES		TIME	MOISTURE	TOTAL CARBOHYDRATES	
	PERCENTAGE FRESH WEIGHT	MG. PER 100 GM. FRESH WEIGHT	PERCENTAGE DRY WEIGHT		PERCENTAGE FRESH WEIGHT	MG. PER 100 GM. FRESH WEIGHT	PERCENTAGE DRY WEIGHT
	%	*mg.*	%		%	*mg.*	%
Aug. 10				Aug. 30			
7 A.M.				7 A.M.			
C.†	81.49	3601	19.41		78.80	4864	18.54
S.‡	80.11	3143	15.78		77.35	4399	18.85
10 A.M.				10 A.M.			
C.	80.50	4305	22.00		73.25	5460	20.92
S.	78.47	3947	18.32		76.80	4975	21.52
1 P.M.				1 P.M.			
C.	76.74	5165	22.10		74.00	5885	22.64
S.	75.71	3920	16.49		75.35	5100	20.73
4 P.M.				4 P.M.			
C.	75.67	4977	20.20		72.50	6425	23.58
S.	73.88	4385	16.80		75.00	5175	20.67
7 P.M.				7 P.M.			
C.	77.28	4742	19.76		74.30	5435	22.38
S.	76.11	4169	18.49		76.70	5235	21.38
10 P.M.				10 P.M.			
C.	79.52	3433	15.81		74.15	5850	22.65
S.	78.36	3072	14.20		74.84	5635	22.40
Aug. 11				Aug. 31			
1 A.M.				1 A.M.			
C.	78.41	3503	15.32		75.35	5330	21.66
S.	81.07	2627	15.52		75.70	5595	23.00
4 A.M.				4 A.M.			
C.	79.62	3329	16.34		77.50	5126	22.87
S.	78.42	2756	12.77		77.80	4808	21.89
7 A.M.				7 A.M.			
C.	79.77	3366	16.65		78.20	4703	21.55
S.	78.94	2844	13.50		76.10	5425	22.67

* The term "total carbohydrates" as used here includes all materials in the leaf which yield reducing substances after acid hydrolysis.

† C = Control plants.

‡ S = Sprayed plants.

The leaf-punch data are shown in table I and in figures 1 and 2. The estimation of total carbohydrates after acid hydrolysis yielded results in accordance with those obtained by the more precise methods of analysis for the sugars. Although the leaf-punch samples showed some erratic fluctuations when the data were calculated on the dry weight basis, the controls usually exceeded the sprayed plants in percentage composition of total carbohydrates.

In the discussion which follows, consideration is given only to the data from the alcohol samples, calculated on the fresh weight basis.

Inspection of the data for the August 10 series of collections (tables II, III) reveals that at 7 A.M. the sugar content of the leaves of the control and sprayed plants was essentially the same. At 10 A.M. the sprayed plants con-

TABLE II

REDUCING SUGAR CONTENT OF MAIZE LEAVES

Time	On basis of		Time	On basis of	
	Mg. per 100 gm. fresh weight	Percentage dry weight		Mg. per 100 gm. fresh weight	Percentage dry weight
	mg.	%		*mg.*	%
Aug. 10			Aug. 30		
7 A.M.			7 A.M.		
C.*	280	1.55		295	1.39
S.†	267	1.34		275	1.21
10 A.M.			10 A.M.		
C.	350	1.79		315	1.17
S.	250	1.16		240	1.03
1 P.M.			1 P.M.		
C.	530	2.27		390	1.50
S.	267	1.10		365	1.48
4 P.M.			4 P.M.		
C.	550	2.25		365	1.32
S.	340	1.30		335	1.34
7 P.M.			7 P.M.		
C.	495	2.17		400	1.55
S.	360	1.50		305	1.30
10 P.M.			10 P.M.		
C.	462	2.25		385	1.49
S.	310	1.43		320	1.27
Aug. 11			Aug. 31		
1 A.M.			1 A.M.		
C.	370	1.71		350	1.42
S.	315	1.66		270	1.11
4 A.M.			4 A.M.		
C.	370	1.81		335	1.48
S.	237	1.09		290	1.30
7 A.M.			7 A.M.		
C.	370	1.82		330	1.51
S.	385	1.82		245	1.02

* C = Control plants.
† S = Sprayed plants.

tained 67.80 per cent. of the quantity of total sugars found in the controls while at 1 P.M. the total sugars in the sprayed plants amounted to only 38.90 per cent. of that in the controls. Maximum accumulation of the total sugars in the leaves of the controls occurred at 4 P.M., amounting to 2238 mg. per 100 gm. of fresh tissue. The maximum total sugar content of the leaves of sprayed plants was 1390 mg. per 100 gm. fresh weight which was 62.10 per cent. of the quantity found in the control leaves. After 4 P.M. a somewhat more rapid decline in the sugar content was shown by the controls than by

the sprayed plants. This was to be expected since the photosynthetic activity in the treated plants was limited and the sugar gradients were steeper in the controls which probably is a factor in translocation from the photosynthetic area.

The reducing sugar content showed some fluctuation in both the control and sprayed plants.

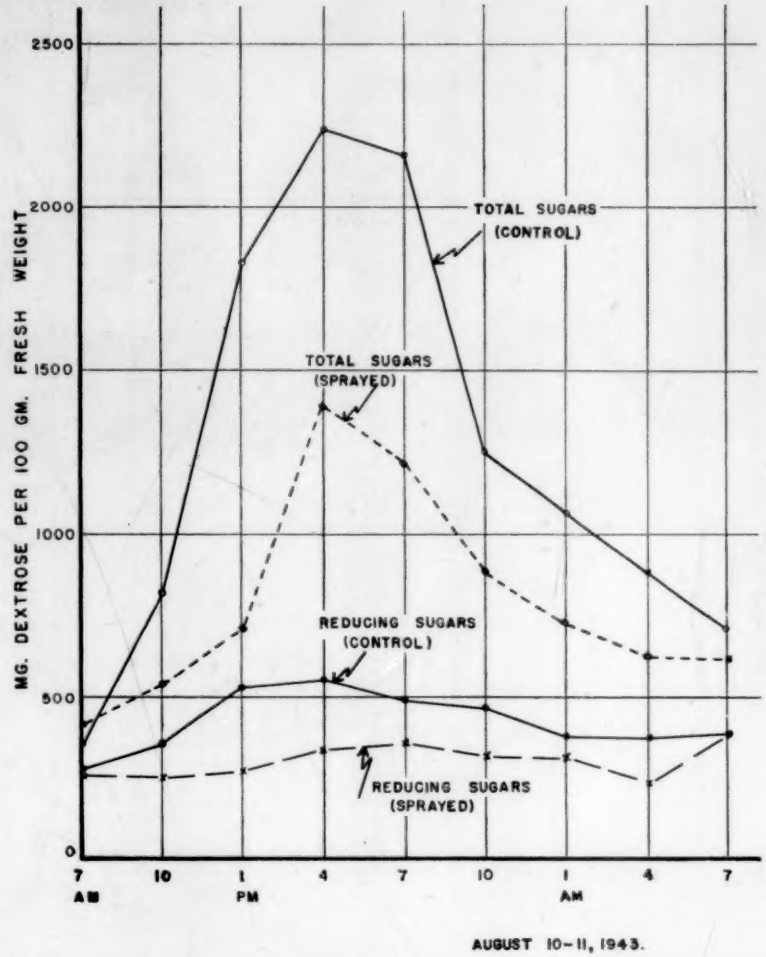

FIG. 3. Variation in the reducing and total sugar content of maize leaves. August 10–11, 1943.

The results showed a maximum reducing sugar content of 550 mg. per 100 gm. tissue in the control plants at 4 P.M. which was coincident with the maximum total sugar accumulation. A slight but apparent lag in the reducing substances was shown by the sprayed plants. This, however, was not observed in the collection of August 30. Compare figures 3 and 4.

The greatest percentage difference in reducing sugars occurred at 1 P.M.

At that time the reducing sugar content of the sprayed plants was 50.2 per cent. of that found in the controls.

The trend of the sugar content in the leaves of the controls and sprayed plants on August 30 (fig. 4) closely resembled that shown at the earlier date. The reducing sugars were uniformly low, the leaves of the control plants containing slightly greater quantities than the leaves of the sprayed plants.

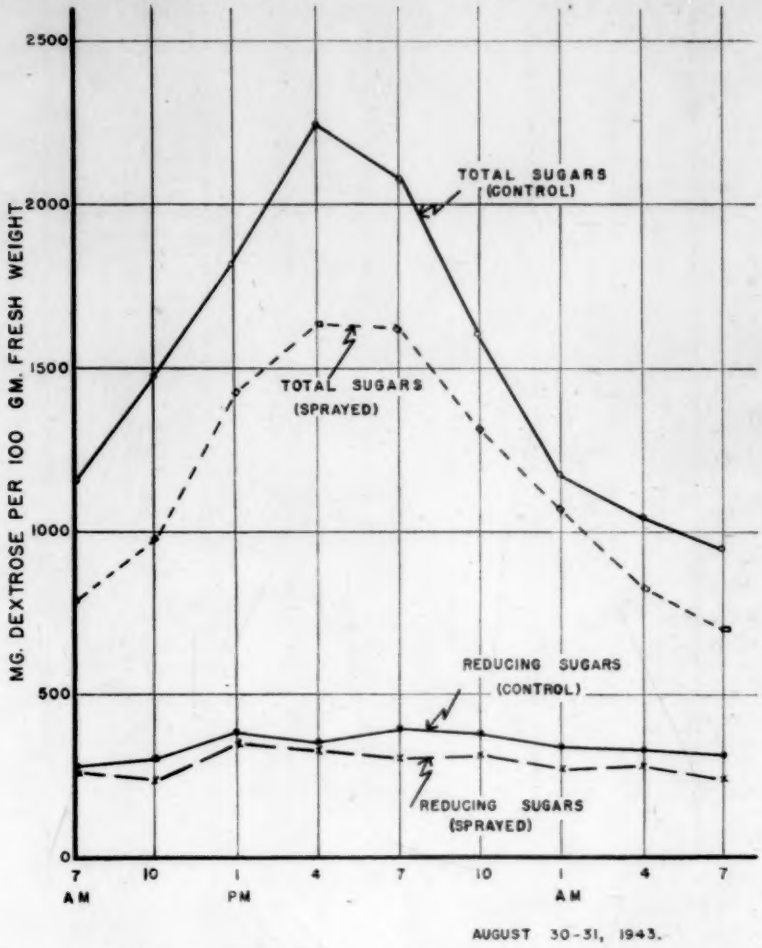

FIG. 4. Variation in the reducing and total sugar content of maize leaves. August 30–31, 1943.

No important variation occurred and the amount present did not exceed 400 mg. per 100 gm. of the fresh tissue.

By August 30 the leaves of the sprayed plants were streaked and discolored which indicated that the emulsion had caused some injury to the chlorophyll. Chlorophyll analyses showed that the controls contained 3.08 mg. chlorophyll per gm. of fresh tissue while the sprayed plants contained 1.48 mg. per gm.

In spite of the presumably reduced rate of carbon assimilation because of a lowered active chlorophyll content, the accumulation of photosynthate in the leaves of the sprayed plants was comparatively greater than at the time of the earlier study. If we assume accumulation of soluble carbohydrates in the leaves to be a suitable index, or measure, of carbon assimilation some rather pertinent meaning may be attached to the data.

It appears that other factors were more effective in controlling the rate of photosynthesis than chlorophyll content. Since the maximum soluble

TABLE III

TOTAL SUGAR CONTENT OF MAIZE LEAVES

TIME	ON BASIS OF		TIME	ON BASIS OF	
	MG. PER 100 GM. FRESH WEIGHT	PERCENTAGE DRY WEIGHT		MG. PER 100 GM. FRESH WEIGHT	PERCENTAGE DRY WEIGHT
	mg.	%		*mg.*	%
Aug. 10			Aug. 30		
7 A.M.			7 A.M.		
C.	360	1.99		1160	5.46
S.	415	2.09		790	3.48
10 A.M.			10 A.M.		
C.	784	4.04		1480	5.53
S.	532	2.47		980	4.22
1 P.M.			1 P.M.		
C.	1825	7.84		1820	7 00
S.	710	2.93		1440	5.84
4 P.M.			4 P.M.		
C.	2238	9.18		2250	8.18
S.	1390	5.31		1640	6.56
7 P.M.			7 P.M.		
C.	2154	9.48		2080	8.09
S.	1205	5.02		1630	6.99
10 P.M.			10 P.M.		
C.	1250	6.10		1610	6.23
S.	880	4.06		1320	5.06
Aug. 11			Aug. 31		
1 A.M.			1 A.M.		
C.	1055	4.88		1180	4.78
S.	735	3.88		1060	4.41
4 A.M.			4 A.M.		
C.	885	4.34		1050	4.66
S.	635	2.94		830	3.74
7 A.M.			7 A.M.		
C.	714	3.53		950	4.36
S.	610	2.89		700	2.92

carbohydrates in the control plants were approximately the same on August 10 and on August 30, it becomes permissible to make a comparison of the performance of the sprayed plants on the two dates. The maximum total sugars in the sprayed plants on August 30 exceeded the maximum reached on August 10 by about 18 per cent. If this increase can be attributed to a more rapidly available supply of carbon dioxide, it suggests that there was probably a change in the permeability of the waxy covering to carbon dioxide during the 20-day interval from August 10 to August 30.

The data may, therefore, be interpreted to mean that under the conditions of this experiment the bulk of the effect of the spray on the photosynthetic process may be attributed to a reduction in the rate of carbon dioxide absorption and not to a reduction in the active chlorophyll content.

That a waxy coating may interfere with the process of gaseous exchange

TABLE IV

TEMPERATURE AND TOTAL SOLAR RADIATIONS*

TIME	TEMP.	GM. CAL. PER CM.²	TIME	TEMP.	GM. CAL. PER CM.²
	°F.	*Hourly totals*		°F.	*Hourly totals*
Aug. 10			Aug. 30		
7:00 A.M.	66.0	0.2	7:00 A.M.	59.5	
8:00 "	66.0	6.4	8:00 "	62.5	2.6
9:00 "	70.0	22.1	9:00 "	68.0	14.3
10:00 "	73.0	38.5	10:00 "	72.0	28.3
11:00 "	76.0	53.9	11:00 "	74.0	44.9
12:00 NOON	78.5	66.0	12:00 NOON	78.0	56.1
1:00 P.M.	81.5	29.5	1:00 P.M.	79.5	57.3
2:00 "	82.0	77.2	2:00 "	82.0	58.2
3:00 "	84.0	77.6	3:00 "	83.0	63.4
4:00 "	84.5	71.2	4:00 "	83.5	57.1
5:00 "	85.0	60.9	5:00 "	82.0	36.5
6:00 "	84.5	46.8	6:00 "	82.0	23.3
7:00 "	84.0	30.1	7:00 "	82.0	22.4
8:00 "	84.0	13.0	8:00 "	78.0	7.1
9:00 "	82.0	2.8	9:00 "	74.0	0.6
10:00 "	73.0	0.5	10:00 "	71.5	0.2
11:00 "	70.0	Daily	11:00 "	70.0	Daily
12:00 M.T.	71.0	total 596.7	12:00 M.T.	70.0	total 472.9
Aug. 11			Aug. 31		
1:00 A.M.	68.5		1:00 A.M.	68.0	
2:00 "	68.0		2:00 "	66.0	
3:00 "	64.0		3:00 "	66.5	
4:00 "	64.0		4:00 "	66.5	
5:00 "	64.0		5:00 "	65.5	
6:00 "	63.0		6:00 "	65.0	
7:00 "	62.5		7:00 "	66.0	

* Solar radiation data were obtained by the use of an Eppley Pyrheliometer and L. & N. potentiometer.

cannot be questioned. Hence if the stomatal openings become covered or plugged, the rate of carbon dioxide absorption is retarded and reduction in the rate of photosynthesis would be expected.

It was observed that the maximum total sugar content in the controls was practically the same on both dates. The rate of accumulation from 7 A.M. to 4 P.M. and the rate of decrease after 4 P.M. until the following morn-

ing was considerably greater on August 10 than on the 30th. It follows, therefore, that the factor or factors which limited the accumulation of soluble carbohydrates were effective on both dates. From the weather data shown in table IV we can discount incident energy as a factor limiting the rate of photosynthesis. It is probable that light was adequate for maximum carbon assimilation on either day although the daily total differed by 123 gm. cal. per sq. cm. Inspection of table IV reveals that from 10 A.M. to 7 P.M. on August 10 the hourly temperatures were uniformly 1 to 3° F. higher than on August 30. Only at 2 P.M. were the temperatures the same on the two days. The data of table III show, however, that the total sugars were not higher but lower on August 10. It appears then that temperature was not the controlling factor.

Water as a raw material in the photosynthetic process is seldom likely to limit the rate of carbon assimilation, unless of course, the supply is not sufficient to take care of transpirational losses and this was not the case in these experiments.

The rate of photosynthesis observed in the control plants in our experiments seems to have been governed by either (a) the supply of carbon dioxide or by (b) the accumulation of the end products. The immediate end products of photosynthesis are usually assumed to be some carbohydrate and oxygen. Accumulation of carbohydrates may in turn be conditioned by the absence of a storage organ (**8**) or a poorly developed translocating mechanism. Since on August 30 the fruit was in the early roasting-ear stage of development, it would be expected that translocation to the ear would have been sufficiently rapid for efficient removal of the sugars from the leaves.

Suitable methods are not available to determine the absolute rate of translocation and photosynthesis separately. The measurement of carbohydrate accumulation in the leaves indicates only that the production of carbohydrates during a given period of time exceeded the rate of their transport and utilization. The rate of translocation may be altered by a number of factors among which are some that are variable such as temperature (**4**). It is conceivable that a balance may be struck between translocation and photosynthesis where the effect of temperature variations on the net result of the two processes cannot be detected by the measurement of the accumulation.

Admittedly this may have occurred in our experiments but it seems more probable that the rate of apparent photosynthesis in the control plants on August 10 and August 30 was limited either indirectly through translocation or because of a restricted supply of carbon dioxide. If accumulation is assumed to be the chief contributing factor in this respect, the failure in the removal of carbohydrates from the leaves could best be explained on the grounds that the capacity of the translocating machinery was inadequate to meet the requirements for the rapid movement of organic solutes.

In defoliation experiments on corn, LOOMIS (8) concluded that with a relatively large leaf area for a given number of developing ovules, translocation probably became a limiting factor in grain development.

In the absence of convincing evidence that the translocating mechanism is normally inadequate it is suggested that the principal factor limiting the rate of photosynthesis in our control plants was available carbon dioxide.

Summary

A study was made of the influence of a transpiration-reducing emulsion on the daily variations in the soluble carbohydrate content of Maize leaves. Using accumulation of sugars in the leaves as a measure of photosynthetic activity, the maximum accumulation of sugars in the sprayed plants on the day following application of the spray was 62.10 per cent. of that in the controls. Analysis of samples collected twenty days after treatment showed that the total sugar content of the leaves of the sprayed plants was about 18 per cent. greater than at the time of the earlier study.

The relatively low rate of apparent photosynthesis in the sprayed plants probably can be attributed largely to a limited supply of carbon dioxide. The increased rate of production of carbohydrates shown by the sprayed plants on August 30 compared with the rate on August 10 may be explained in either one or both of two ways. A change in the permeability of the waxy covering to carbon dioxide may have occurred during the 20-day interval. Carbon dioxide may also have become more readily available as a result of shrinking or cracking of the spray film.

Although conclusive data are not available, the most reasonable inference from the results is that the normal supply of carbon dioxide to the photosynthesizing cells of the control plants was not adequate to meet the requirements for maximum photosynthesis under the existing conditions.

The author is indebted to W. U. GARSTKA, Project Supervisor, of the Soil Conservation Service, U. S. Department of Agriculture, who supplied the data on solar radiation and temperature. Aid by STEPHAN DJANG is acknowledged in the collection of the samples and in the analysis for sugars. Thanks are due E. J. BENNE and DOROTHY ROSE of the Agricultural Chemistry Department for making the chlorophyll determinations.

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DIURNAL AND SEASONAL CHANGES IN THE ASCORBIC ACID CONTENT OF SOME VEGETABLES[1]

HANS PLATENIUS

(WITH THREE FIGURES)

Introduction

As part of a general study concerned with improving the food value of crop plants, a number of experiments have been conducted to determine the factors which control the ascorbic acid content of plants. The most important papers on this subject have been reviewed by REID (**6**) and more recently by MAYNARD and BEESON (**3**). In spite of some inconsistencies in their results, these investigations lead to the following general conclusions:

1. Mineral nutrition has little influence on the ascorbic acid content of plants except under conditions where normal growth is seriously disturbed by deficiencies in one or more essential elements.

2. Genetic factors play an important rôle. Different varieties of the same species frequently show large differences in the quantity of vitamin C they contain.

3. Some data indicate that the growing temperature affects the rate at which ascorbic acid accumulates in the plant. MOLDTMAN (**4**) and REID (**5**) observed that at moderate temperatures an inverse relation exists between the ascorbic acid content of plants and the temperature at which they are grown.

4. Light, its intensity and the duration of illumination, are known to have a definite influence on the vitamin C content of plant tissue. REID (**6**) studied the effect of various light intensities on cowpea seedlings. SUGAWARA (**8**) exposed etiolated seedlings of several kinds of vegetables to artificial light of different intensities. HAMNER and PARKS (**2**) determined ascorbic acid in turnip greens grown for seven days at light intensities ranging from 200 to 5000 foot candles. All these studies show that, other conditions remaining unchanged, the ascorbic acid of plant tissue increases with rising intensities of light. The magnitude of the increase depends on the duration of the light treatment, the range of intensity studied, and the type of plant tissue involved.

A question of practical importance is whether an appreciable decrease occurs in the ascorbic acid content of crop plants at night. MOLDTMAN (**4**) observed diurnal fluctuations in the vitamin C content of leaves of deciduous trees which in some instances varied as much as 50 per cent. However, since MOLDTMAN expressed his data as percentages of fresh weight, part of the observed fluctuations were probably caused by corresponding diurnal changes in the water content of the leaves. REID (**6**) working with seedlings of cowpea plants found a significant rise in the absolute quantity of vitamin

[1] Paper no. 264 Department of Vegetable Crops, Cornell University, Ithaca, N. Y.

C per plant toward early afternoon. Expressed as percentages of dry weight diurnal fluctuations were less than 10 per cent. and the trend was not always consistent.

All these experiments emphasize the importance of light intensity as a factor controlling the ascorbic acid content of plants. A question which remains to be solved is whether under field conditions diurnal periods of light and darkness or periods of cloudy weather have an effect on ascorbic acid large enough to justify changes in the customary procedures of harvesting crop plants.

Materials and methods

Six different vegetables were used in this study: snap beans (*Phaseolus vulgaris*, var. *humilis*); sprouting broccoli (*Brassica oleracea*, var. *italica*); cauliflower (*Brassica oleracea*, var. *capitata*); kale (*Brassica oleracea*, var. *acephala*); spinach (*Spinacia oleracea*); and Swiss chard (*Beta vulgaris*, var. *cicla*). With the exception of snap beans and Swiss chard these vegetables are good sources of vitamin C. The samples were obtained from healthy, vigorously growing plants. In order to minimize errors due to variability among the individual plants, samples of cauliflower and broccoli were taken on successive days from the same head of a single plant. Of the other vegetables not less than 20 leaves or fruits were harvested from an equal number of plants. To obtain comparable samples of kale, which was sampled repeatedly from the same plants over a long period, only leaves near the growing point, measuring 2 to 3 inches in length, were taken.

Within one-half hour after harvest each composite sample was cut up quickly into small sections, mixed thoroughly, and triplicate portions were weighed out for analysis and determination of dry weight.

The method of analysis for ascorbic acid was essentially the same as that devised by Bessey and King (1). Five- or ten-gram samples were extracted with a mixture of one per cent. normal sulphuric acid and two per cent. metaphosphoric acid. A Waring blender served to macerate the tissue. Titrations were made against dichlorophenol-indophenol which was standardized against pure ascorbic acid.

Dry matter was determined by drying the samples in a ventilated oven at 70° C. for 48 hours.

Temperature records were obtained from thermograph records at a level of two feet above ground. The data for solar radiation were calculated from the records of a pyroheliometer which measured light intensities in terms of gram calories per square centimeter. The instruments for recording temperature and solar radiation were located at a distance of about 3 miles from the place where the vegetables used in this study were grown.

Results

In order to determine diurnal changes in the ascorbic acid content of vegetables, samples were taken late in the afternoon between five and seven

o'clock (standard time) and the following morning between six and seven o'clock. Aside from a few exceptions, which will be noted later, sampling was done on a clear day during which the plants received at least ten hours of full sunlight.

The results of ascorbic acid analyses of four different vegetables harvested in the evening and the following morning are given in table I. Expressed on a fresh weight basis, the data show a small, but consistent decrease in ascorbic acid content during the night. Determinations for changes in the percentage of dry weight showed a higher water content of the plant tissue in the morning than in the evening. This difference was

TABLE I

DIURNAL CHANGES IN ASCORBIC ACID AND DRY MATTER OF VEGETABLES

VEGETABLES	TIME OF HARVEST	DRY MATTER	PERCENTAGE OF ASCORBIC ACID	
			FRESH WT.	DRY WT.
		%	%	%
Broccoli	7/15 7 P.M.	14.63	1.485	10.15
	7/16 6 A.M.	12.12	1.210	9.98
	7/16 6 P.M.	15.16	1.480	9.76
	7/17 6 A.M.	13.66	1.340	9.81
Broccoli	8/2 5 P.M.	15.40	1.567	10.18
	8/3 6 A.M.	13.14	1.329	10.11
Snap beans	7/18 6 P.M.	8.34	0.290	3.48
	7/19 6 A.M.	7.93	0.286	3.61
Cauliflower	7/27 7 P.M.	9.32	0.852	9.14
	7/28 7 A.M.	8.70	0.737	8.47
	7/30 7 P.M.	8.20	0.770	9.39
	7/31 7 A.M.	7.77	0.704	9.06
Swiss chard	8/31 5 P.M.	11.26	0.182	1.62
	9/1 7 A.M.	9.82	0.178	1.81

due partly to slight wilting after exposure to full sunlight and partly to the presence of dew in the morning. After making corrections for these fluctuations in water content by calculating the data for ascorbic acid on a dry weight basis, no consistent differences in vitamin C content could be detected except for cauliflower. Even in cauliflower the loss in vitamin C during the night was less than eight per cent. It should be noted that the data are expressed on a percentage basis. It is possible that because of growth and translocation the changes in absolute quantities of ascorbic acid per plant were somewhat different from those recorded in table I. Unfortunately, the method of sampling used did not permit the calculation of ascorbic acid values on an absolute basis.

According to observations by REID (**6**) leaf tissue responds more readily to changes in light intensity than other parts of the plant. For that reason an experiment was conducted in which the ascorbic acid content of kale leaves was recorded consecutively at 6 A.M. and 6 P.M. for a period of five days. These data, expressed as percentages of dry weight are presented in

figure 1 together with corresponding values of solar radiation and temperature recorded at two- and three-hour intervals, respectively. Again the results failed to show consistent variations between samples taken in the morning and evening. The slight rise in the level of ascorbic acid beginning September 14, may reflect the result of a continual exposure to bright sunlight for three days. It is doubtful, however, whether the small difference was larger than the experimental error.

A similar experiment, carried out with spinach, afforded a good opportunity to study the relation between changing light intensities and the ascorbic acid content of the leaves. The results are shown in figure 2. The

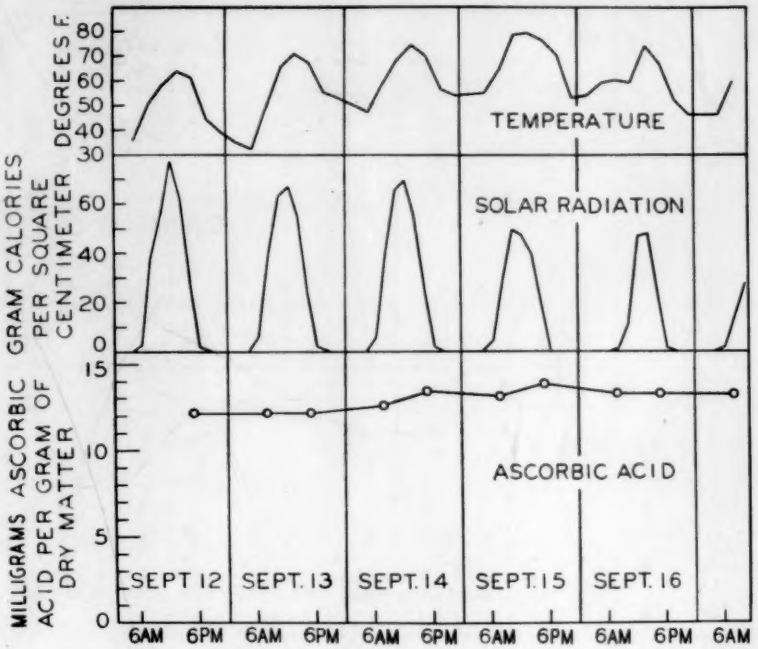

FIG. 1. Relation between temperature, solar radiation and ascorbic acid content of kale, sampled twice daily for a five-day period.

weather was clear during the first and third day, partly cloudy during the second and fourth, and it rained all day on the fifth day. At the same time the average daily temperature dropped about 23° F. during this period. The concentration of ascorbic acid declined slightly on August 24, the second day of the experiment, rose again the following day, and remained at a uniform level until the end of the experiment. A comparison of these data with corresponding values of light intensity may lead one to attribute the decline in ascorbic acid on August 24 to the fact that it was partly cloudy during that day. However, three days later, when on August 27, solar radiation was at an extremely low level, ascorbic acid values remained practically the same as on the preceding day, thus making it extremely doubtful

whether a single cloudy day has a depressing effect on the ascorbic content of spinach leaves. It is not impossible, however, that the relatively low temperature prevailing August 27 completely compensated for any depressing effect that the low light intensity may have had.

Although the foregoing experiments make it appear unlikely that single days of cloudy weather have an appreciable effect on the ascorbic acid of leafy vegetables, the possibility exists that differences in light intensities maintained over longer periods of time bring about such an effect. In order to clarify this question, the ascorbic acid content of kale leaves was determined eight different times during the period from July to December. The

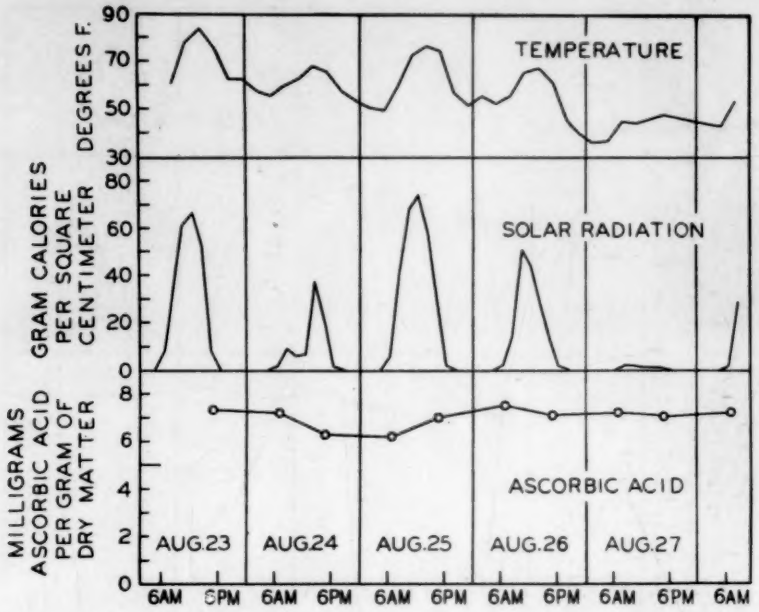

FIG. 2. Relation between temperature, solar radiation and ascorbic acid content of spinach sampled twice daily for a five-day period.

results may be influenced to some extent by the fact that the plants, from which the samples were taken, increased about three-fold in size during this period. To eliminate this age factor as far as possible, only young leaves of approximately the same size were harvested. All samples were taken at 6 A.M. Weekly averages were calculated for temperature and total daily radiation. The data from ascorbic acid analysis were expressed as percentages of dry matter.

A general downward trend in vitamin C values throughout the season is apparent; it follows the same general trend as the decline in solar radiation (fig. 3). The abrupt drop in light intensity during the middle of October was followed a week later by a sudden decline in ascorbic acid content. A positive correlation between light intensity and concentration of ascorbic acid in the leaves was to be expected on the basis of earlier investigations.

What proved to be surprising was the fact that the total decline in vitamin C between July and October was only about 20 per cent. in spite of the fact that the total solar radiation decreased about 76 per cent. during this period. Perhaps the corresponding drop in average temperature from 72° to 41° F. was sufficient to counteract in part the effect of declining solar radiation.

Although the samples consisted of young leaves of approximately the same size, it is possible that part of the seasonal decline in ascorbic acid of

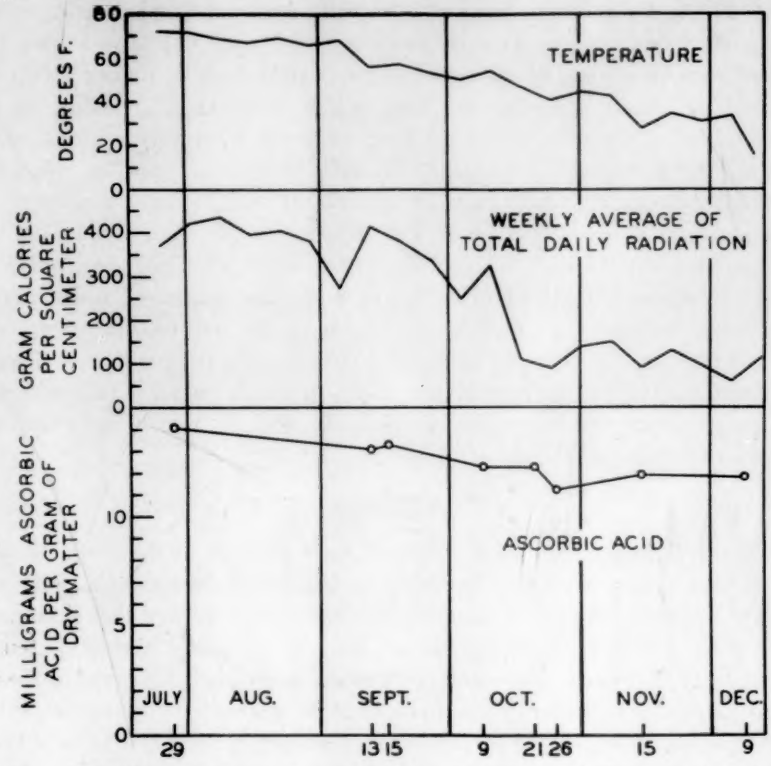

FIG. 3. Relation between weekly average temperature, weekly average of total daily radiation and ascorbic acid content of kale for the period beginning July 29 and ending December 9.

kale can be attributed to the advancing stages in the maturity of the plants. If such a correlation exists it would tend to minimize still further the effect of decreasing light intensities.

Discussion

Based on earlier investigations concerning the effect of light intensity on the ascorbic acid content of plants the suggestion has been made that crop plants be harvested whenever possible following periods of clear, sunny weather. Some have advocated that the picking of vegetables be delayed until the afternoon hours of the day of harvesting, at which time the plants are supposed to have reached their highest content of vitamin C.

The results of the study described in this paper indicate that under field conditions the effect of variable light conditions on the ascorbic acid content of vegetables is small. No diurnal fluctuations in vitamin C content could be detected and single days of cloudy weather were without effect.

There are several reasons which may explain why the studies of MOLDTMAN (**4**), REID (**7**), and others led to different conclusions. Some of the earlier studies were carried out during the winter in the greenhouse, others were made under artificial light at intensities considerably below those prevailing outdoors. REID (**6**) and HAMNER and PARKS (**2**) have shown that the response to variations in light intensity decreases as higher levels are approached. Consequently, one can expect only small changes in the ascorbic acid content of plants growing outdoors where on bright summer days the light intensity frequently exceeds 10,000 foot candles. Another factor influencing the magnitude of changes in ascorbic acid is the type of plant material used. REID (**5, 6, 7**), MOLDTMAN (**4**), and SUGAWARA (**8**) used seedlings in their studies which, according to REID (**6**), respond more readily to changes in light intensity than do the tissues of older plants. Even more important is the fact that plants under field conditions are subject to simultaneous changes in solar radiations and temperature. During the summer months, periods of low light intensity usually coincide with periods of relatively low temperatures, two factors which have opposite effects on the level of vitamin C in plants.

Summary

Diurnal fluctuations of ascorbic acid were studied in six kinds of vegetables: snap beans, sprouting broccoli, cauliflower, kale, spinach, and Swiss chard. Expressed on a fresh-weight basis the results showed that these vegetables when harvested in the late afternoon had a slightly higher content of ascorbic acid than those harvested early in the morning. However, these differences were almost entirely due to diurnal fluctuations in the water content of the plants. Calculated as percentages of dry weight the results failed to show consistent differences between morning and evening samples.

The ascorbic acid content of kale and spinach determined at 6 A.M. and 6 P.M. for a period of five days remained remarkably constant. A single day of cloudy, rainy weather had no depressing effect on the vitamin C content of spinach. The concentration of vitamin C in leaves of kale declined about 20 per cent. from July 29 to October 26. Weekly averages of total daily radiation during this period decreased about 76 per cent. It is suggested that a corresponding drop in the average temperature counteracted in part the effect of lowered light intensity.

The writer is indebted to DR. R. BRADFIELD of the Agronomy Department for making available the temperature records and to DR. A. J. HEINICKE of the Department of Pomology for data on solar radiation.

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GROWTH AND BIOCHEMICAL COMPOSITION OF BEAN PLANTS AS CONDITIONED BY SOIL MOISTURE TENSION AND SALT CONCENTRATION

C. H. WADLEIGH AND A. D. AYERS

(WITH NINE FIGURES)

There is evidence in the literature that decreasing soil moisture content is associated with increases in the osmotic pressure of the tissue fluids in both roots and tops of plants (**26, 27**), decreases in rate of vegetative growth (**1, 9, 15, 30, 64**), modifications in stomatal opening (**16, 17**), a depletion in starch reserves (**15, 35, 52, 64**), a decrease in apparent photosynthesis, and an increase in respiration (**50**). Many of these effects were noted even though the respective plants did not wilt, and notwithstanding the fact that there is considerable evidence (**8, 24, 58, 62**) that all soil moisture above the wilting percentage (that held by less than 15–18 atmospheres of tension) is equally available to plants, and that variations in this range of available moisture are without effect on plant growth.

Most of the foregoing observations were made on non-saline soils. There appears to be little information available as to the effect of increasing salt concentration in the soil solution upon plant metabolism. The best work bearing on this relationship is that of NIGHTINGALE and FARNHAM (**37**). They grew sweet peas in sand culture with complete nutrient solutions varying in osmotic pressure with the purpose of inducing "physiological drought." They presented their data on the green weight basis and found that the content of sugars and starch in the plants increased with an increase in the osmotic pressure of the substrate. By contrast, both the soluble organic nitrogen and the protein contents of the plants decreased, even though there were ample amounts of nitrate nitrogen present in the tissues. These authors suggested that "protein starvation" was actually involved in the reductions in meristematic activity and in vegetative growth accompanying increased concentration of the culture solution.

WADLEIGH and GAUCH (**63**) grew Red Kidney beans in water culture in which high concentrations of $CaCl_2$ and NaCl were added to the base nutrient and assayed the plants for certain nitrogenous and carbohydrate constituents. They found a progressive diminution in the concentration of NO_3–N in the tissues with increase in osmotic pressure of the substrate in the presence of either salt. Percentage of protein, especially in the leaves, also decreased with increasing salt concentration, whereas percentage of soluble organic N was practically unaffected. The plants were grown at a time of year (December) when the daily quantity of solar radiation was low and, as a consequence, the carbohydrate level in the plants was relatively low. However, increasing the salt concentration of the culture medium was associated with general increases in both reducing and nonreducing sugars in all parts of the plants (leaves, stems, and roots). None of the plants subjected

to saline treatments showed any definite starch accumulation above that of the control plants. Those grown on the lower concentrations of added salt were found to have a definitely lower percentage of starch than the controls; but, within saline cultures, percentage of starch tended to increase with increased osmotic pressure of the culture solution. In comparing the results of these two investigations it should be stressed that NIGHTINGALE and FARNHAM (**37**) increased the osmotic pressure of their nutrient solutions by uniformly increasing all constituents, thereby effecting a large increase in nitrate supply; whereas WADLEIGH and GAUCH (**63**) maintained the nitrate level constant and increased the osmotic pressure by additions of chloride salts only. In the latter study, the high concentrations of the chloride ion may have been somewhat inhibitory to carbohydrate synthesis. Heavy applications of chloride salts in fertilizers have been found to decrease the chlorophyll content of potato leaves (**5, 41**) with a concomitant decrease in the total carbohydrate content of the leaves (**4**).

A previous report (**3**) from this laboratory presented data on the yield of Red Kidney bean plants subjected to varying degrees of salt concentration and soil moisture tension. The wide range in yields observed was regarded as indicative of a similar range in the metabolic status of the plants during the period of active vegetative growth.

It follows that the effect of soil salinity upon crop yields can be better interpreted with the advent of increasing information concerning the effect of saline substrates on specific metabolic processes within the plant. Consequently, the above-mentioned experiment (**3**) was repeated with the exception that the plants were harvested at incipient florescence and assayed for a few of the biochemical constituents important in plant metabolism.

Experimental procedure

Thirty-six steel drums with a ten-gallon capacity were each filled with Fallbrook loam soil. Determinations on a pressure-membrane apparatus (**46**) indicated that this soil held 6.2 per cent. moisture when moistened and allowed to come to equilibrium under a tension of 15 atmospheres. The permanent wilting percentage determined with sunflower plants was 6.1. The close agreement of these two values corroborates the report by RICHARDS and WEAVER (**46**) that the 15-atmosphere percentage lies in the wilting range. The moisture equivalent of this soil was 14.7 per cent., and the normal moisture capacity 18.0 per cent. (**51**). The tension on the moisture in this soil as a function of percentage of moisture is shown in figure 1. With the moisture content at saturation, the original soil had a pH of 7.6 and a specific conductance of 34×10^{-5} mhos at 25° C. Calcium was the predominating cation in the exchange complex at the start of the experiment, and there were no accumulated soluble salts in the original soil.

The authors are indebted to DR. MILTON FIREMAN for the data on the tension on the moisture in soil as a function of percentage of moisture; such data were secured by methods described elsewhere (**42, 45**). These data are

frequently plotted with a logarithmic scale (log tension in centimeters of water) as the ordinate rather than with the linear scale used in figure 1. The latter presentation emphasizes the hyperbolic nature of this curve; *i.e.*, it accentuates the fact that as moisture content decreased in this soil from 30 to 11 per cent. the tension increased only about one atmosphere; whereas a drop in soil moisture content from 11 to 6 per cent. caused the tension to increase by 14 atmospheres.

The experimental variables were:

I. Salt treatments 4
   a. No added NaCl
   b. 0.1 per cent. added NaCl on dry soil basis
   c. 0.2 per cent. added NaCl on dry soil basis
   d. 0.4 per cent. added NaCl on dry soil basis

II. Irrigation schedules 3
   a. Irrigated when only 40–50 per cent. of the available moisture had been removed from the soil
   b. Irrigated when 60–65 per cent. of the available moisture had been removed from the soil
   c. Irrigated when 90–100 per cent. of the available moisture had been removed from the soil

The drums were randomized within 3 blocks. This gave one drum of each combination of salt and irrigation schedule per block. Following established convention, the available moisture in a soil was taken as that between the field capacity (20 per cent.) and the permanent wilting percentage.

The proper amount of salt for each treatment was mixed with the screened soil ($\frac{1}{4}''$) in a box suspended and rotated eccentrically. Besides the designated sodium chloride, 3.4 grams of potassium chloride and 10.1 grams of 16 per cent. superphosphate were added to each drum of soil. One hundred and seven pounds of the soil at a moisture content of 5 per cent. were placed in each container with the aid of a mechanical mixer and packer. Nitrate was supplied by scratching 13.2 grams of calcium nitrate dihydrate into the soil surface of each drum just before the initial irrigation. These amounts of fertilizing materials corresponded to an application of 1000 pounds of 6–8–6 fertilizer per acre (per 2,000,000 pounds of soil).

Tensiometer cups (**43**) were placed at depths of 4 inches and at 14 inches (bottom) in each drum of one set of replicates. Enough tap water was added to bring the soil to an average moisture content of 20 per cent. This value was identical with that found for the field capacity of this soil. The drums were weighed daily and when the average moisture content reached a designated degree of depletion sufficient tap water was added to reestablish the 20 per cent. level of soil moisture.

On April 4, 1942, after the irrigated soils had been standing for several days, eight germinated dwarf Red Kidney beans were placed in each culture. When the seedlings were well established, they were thinned to the

four most uniform per container. The plants were grown until the first blossoms were beginning to appear and then harvested. This harvest took place between 6:00 and 7:30 A.M. on May 7. Plants in all treatments were turgid at this time. The sky was heavily overcast at this time and a mini-

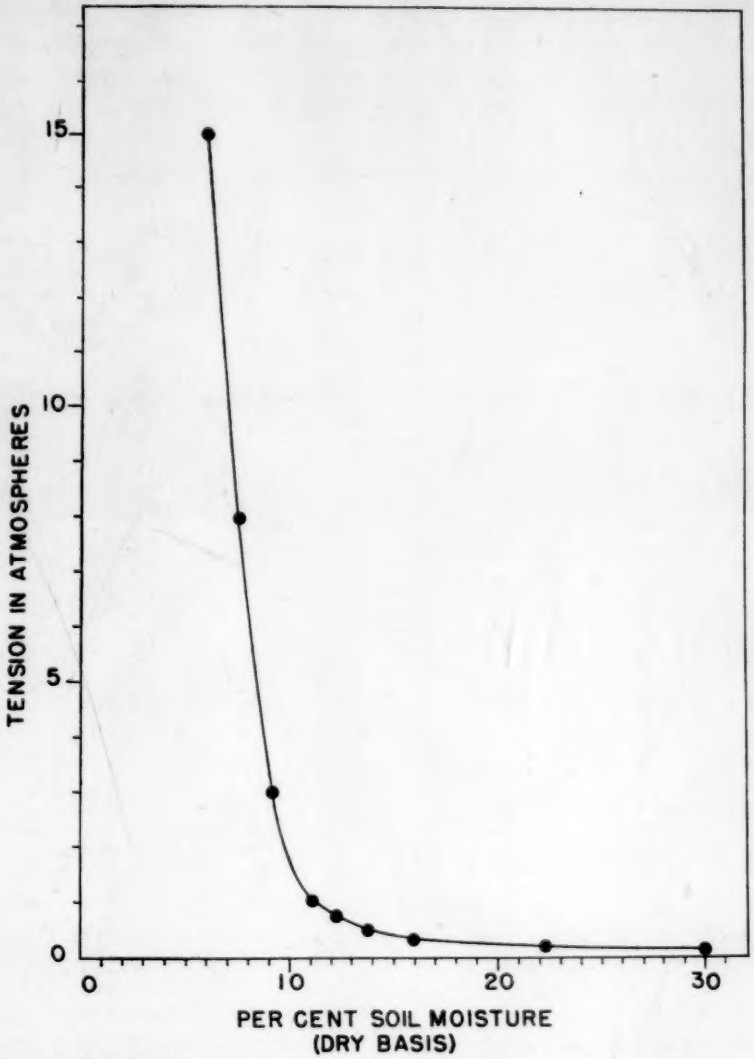

FIG. 1. Relationship between soil moisture percentage and moisture tension of the sample of Fallbrook loam.

mum of change in the relative status of the metabolites was regarded to have taken place during this time interval. On harvesting, each plant was weighed, segregated into (a) leaves, and (b), stems plus petioles, and prepared for preservation by rapid desiccation in a forced draft oven maintained at about 75° C. The dried tissue was ground in a small Wiley mill and then in a ball mill until all tissue would pass a 100-mesh screen.

Total nitrogen including nitrate was determined by a micro-modification of the method of RANKER (**40**). Total soluble nitrogen was extracted according to the procedure described by Clark (**7**), and assayed by the method of PUCHER and VICKERY (**39**) using reduced iron to effect nitrate reduction. Nitrate nitrogen in the extract was determined by a micro-modification of the usual DEVARDA method. Soluble organic nitrogen was taken as the difference between nitrate and total soluble nitrogen. "Protein" nitrogen was calculated as the difference between total nitrogen and total soluble nitrogen.

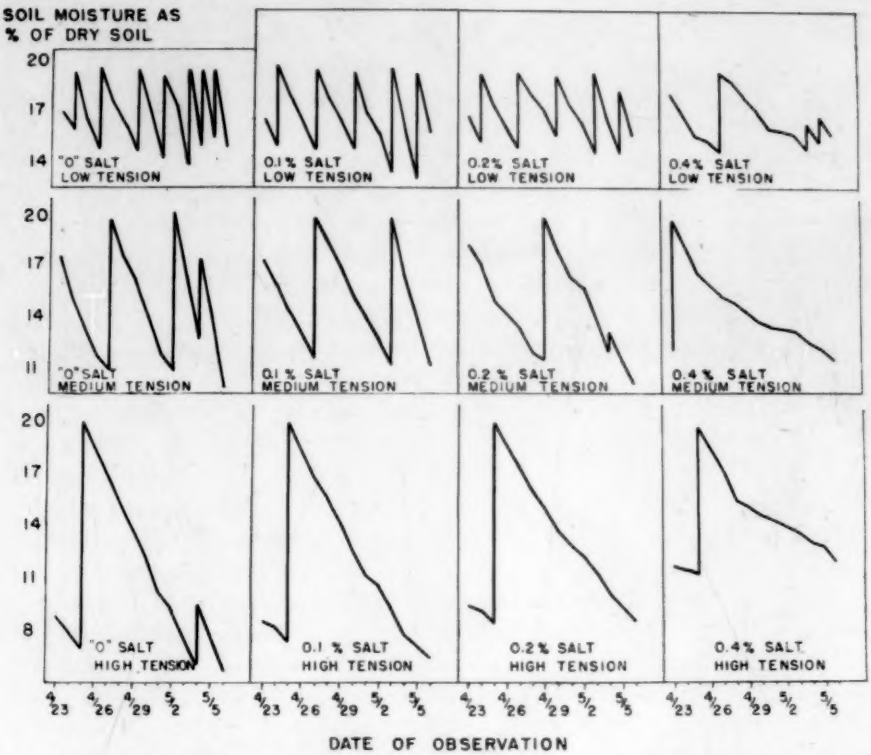

FIG. 2. The range of average soil moisture percentage in the soil of the various treatments for two weeks prior to the harvest.

For sugar determinations, 500 mg. of the powdered tissue was extracted with 100 ml. of 85 per cent. alcohol by refluxing one-half hour. After removing the alcohol from the extract, clearing, and deleading, the reducing and non-reducing sugars were determined by the method of TOMPSETT (**55**). Invertase was used as the hydrolytic agent for sucrose. Starch was determined on the residue following extraction of dextrins with 10 per cent. alcohol by the method described by LOOMIS and SHULL (**31**) using fresh saliva as the hydrolytic agent.

Results

Figure 2 presents the trends of average soil moisture content for each

treatment during the last two weeks of the experimental period. The plants on the "0" salt-"low"-moisture-tension treatment had to be watered nearly every day during the last few days of the study. As the salt content of the soil was increased the frequency of irrigation within a given soil moisture regime decreased. This was due to the fact that higher levels of soil salinity resulted in a corresponding reduction in plant growth, thereby lowering the rate of water removal from the soil. As the degree of soil moisture depletion prior to irrigation was intensified, the frequency of irrigation was also reduced. This reduction in irrigation-frequency was not entirely due

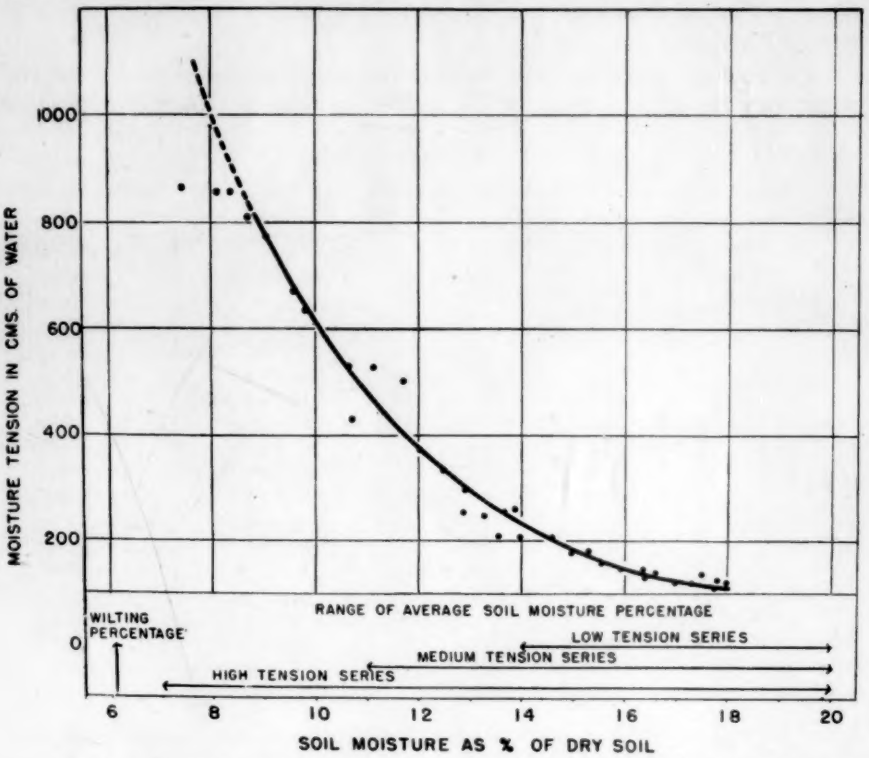

FIG. 3. The relationship between the average soil moisture of the "0" salt treatment and the tension at the four-inch depth.

to the wider ranges in soil moisture content, since the correlated reduction in plant growth resulted in decreased rate of water removal. It was planned that the date of harvest should occur when the soil moisture tension would be approaching the maximum specified for any given treatment. This goal was only partially realized. For example, the moisture levels of the "medium-tension" and "high-tension" treatments in the soil containing 0.4 per cent. salt were so nearly identical prior to the date of harvest that little or no response to differential treatment could be expected.

It may be seen from figure 3 that tensiometer readings (at the 4-inch depth) for various average soil moisture contents in the "0" salt treatments

tended to follow the general trend of that shown in figure 1, but with an appreciable displacement to the left. Tensiometers are not reliable above 800 cm. of tension, thereby accounting for the anomalous observations at the lower moisture percentages. RICHARDS and FIREMAN (**45**) have shown that the locus of a soil moisture sorption curve may be shifted depending on treatment of the soil prior to the determination. Furthermore, it should be emphasized that the soil moisture percentages used in figure 3 are averages for the whole mass of the 100 pounds of soil, whereas the tensiometer values indicate the moisture status of only a portion of the total soil mass in the drum. That is, one could not expect the loci of the respective curves in figures 1 and 3 to coincide.

The average green and dry weights per plant produced under the imposed experimental conditions are shown in table I and the former in

TABLE I

AVERAGE GREEN AND DRY WEIGHTS PER PLANT FROM THE VARIOUS TREATMENTS

TREATMENT		GREEN WT.	DRY MATTER	DRY WT.
SALT LEVEL	MOISTURE TENSION			
%		*gm.*	%	*gm.*
"0"	Low	61.0 ± 2.8*	14.1 ± 0.11	8.62 ± 0.32
"	Med.	51.3 ± 4.1*	13.6 ± 0.09	7.42 ± 0.58
"	High	32.5 ± 1.2	15.8 ± 0.08	5.17 ± 0.20
0.1	Low	46.2 ± 0.8	13.6 ± 0.01	6.30 ± 0.11
"	Med.	35.5 ± 2.1	14.0 ± 0.19	4.95 ± 0.34
"	High	23.7 ± 1.0	15.5 ± 0.08	3.67 ± 0.17
0.2	Low	30.7 ± 2.6	13.9 ± 0.12	4.27 ± 0.38
"	Med.	24.1 ± 0.5	14.7 ± 0.05	3.67 ± 0.05
"	High	18.1 ± 0.2	14.9 ± 0.11	2.70 ± 0.02
0.4	Low	9.6 ± 0.4	14.8 ± 0.23	1.42 ± 0.07
"	Med.	8.4 ± 0.8	14.9 ± 0.08	1.27 ± 0.13
"	High	7.7 ± 0.8	14.8 ± 0.05	1.12 ± 0.10

* All errors given are standard errors.

figure 4. The observed trends not only show the marked reduction in growth resulting from increased soil salinity, but also indicate that the level of response to a given salt content of the soil was modified by the extent of soil moisture depletion prior to irrigation. To be sure, the concentration of the soil solution will increase as the soil dries out but, in a non-saline soil, the pressure potential becomes a more important component of the free energy of the soil moisture than the osmotic potential (**10**). The above responses towards increasing soil moisture tension confirm those previously reported (**3**) for a parallel experiment in which yields of dried beans were used as the criterion of growth.

The term "pressure potential" is sometimes designated as "capillary potential." The moisture potential (**61**) of the soil moisture (free energy per unit mass) was shown by DAY (**10**) to be the summation of (a) the

pressure potential (free energy per unit mass developed by attractive forces between soil particles and surrounding water), and (b) the osmotic potential (free energy per unit mass due to solutes). Tensiometers and pressure-membrane apparatus measure the pressure potential of the soil moisture; the osmotic potential of the displaced soil solution may be determined cryoscopically. A potential function is not dimensionally expressible in terms

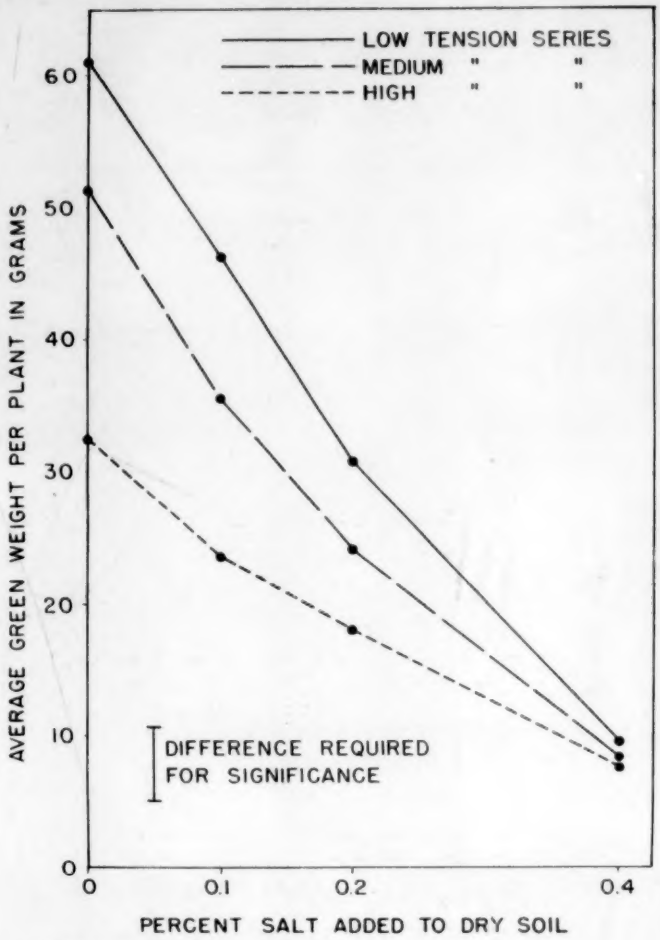

FIG. 4. Average fresh weight of plants.

of atmospheres, but it is conducive to clarity in this report to discuss the forces acting on the soil moisture in terms of atmospheres. A moisture potential of -1×10^6 ergs per gram is approximately equivalent to a pressure of one atmosphere. In line with the work of RICHARDS and WEAVER (**46**), the term "moisture tension" expressed in atmospheres will be used to designate the free energy of the soil moisture involved in the pressure potential, osmotic pressure to designate that of the osmotic potential, and "total mois-

ture stress'' in atmospheres (sum of osmotic pressure + moisture tension) will be used to designate the total free energy of the soil moisture as covered by the term moisture potential.

The variations in percentage of dry matter found in these plants are shown in figure 5. Under the water regimes designated as ''low'' and ''medium'' tension, there was a tendency for the percentage of dry matter to increase with increase in salt content of the substrates, excepting that

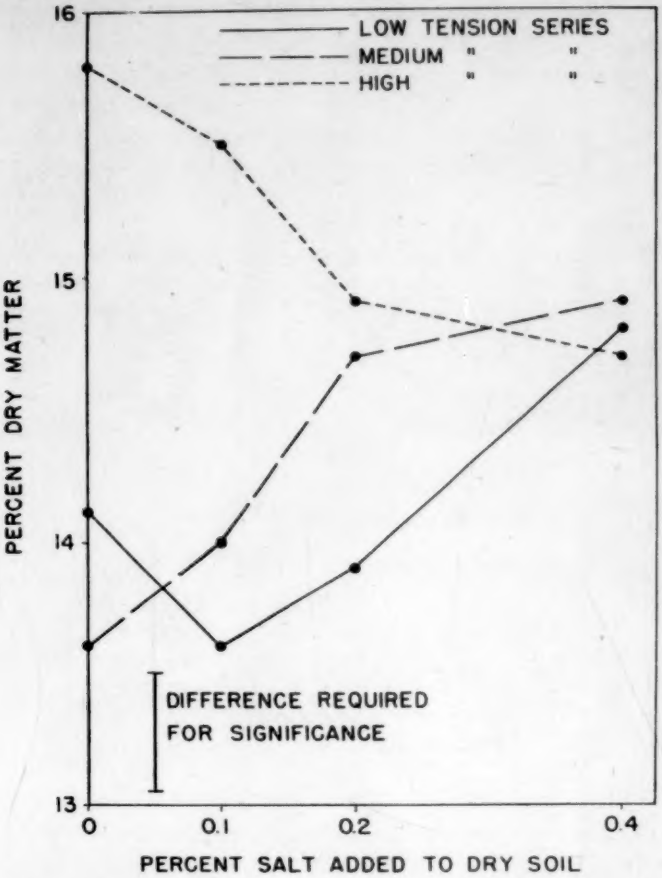

FIG. 5. Average percentage dry matter of plants.

within the ''low'' tension series the ''0'' salt plants had a significantly higher percentage of dry matter than those grown on soil containing 0.1 per cent. salt. In contrast, the plants in the ''high'' tension series showed a definite decrease in percentage of dry matter with increased soil salinity. This is not an unusual finding; another study (**64**) on beans grown in saline soil consistently showed this same trend. In general, the relative positions of the curves in figure 5 tend to show an increased percentage of dry matter with increased degree of soil moisture depletion prior to irrigation, even

TABLE II

BIOCHEMICAL COMPOSITION OF BEAN LEAVES AND STEMS AS PERCENTAGE OF DRY MATTER

Plant part	Treatment		NO_3–N	Sol. org. N	Protein N	Total N	Reducing sugars	Non-reducing sugars	Starch	Hemi-reserves
	Salt level	Moisture tension								
	%		%	%	%	%	%	%	%	%
Leaves	"0"	Low	0.08	0.47	2.94	3.51	0.97	2.46	4.64	5.90
	"	Med.	0.17	0.49	3.50	4.16	0.67	1.46	0.59	5.34
	"	High	0.25	0.61	3.63	4.49	0.81	1.30	0.59	5.18
	0.1	Low	0.08	0.39	3.40	3.77	0.78	1.61	2.76	6.22
	"	Med.	0.13	0.54	3.42	4.09	0.77	1.63	1.29	5.64
	"	High	0.16	0.67	3.58	4.41	0.70	1.65	0.42	5.60
	0.2	Low	0.13	0.49	3.66	4.28	0.45	1.39	2.38	6.08
	"	Med.	0.18	0.59	3.95	4.72	0.47	1.44	0.66	5.80
	"	High	0.17	0.61	3.89	4.50	0.53	1.65	0.60	5.94
	0.4	Low	0.23	0.62	3.82	4.44	0.84	1.23	0.87	5.68
	"	Med.	0.23	0.61	4.09	4.70	0.63	1.36	0.41	5.06
	"	High	0.25	0.67	4.13	4.80	0.78	1.33	0.44	6.02
Stems	"0"	Low	0.12	0.71	1.19	2.02	4.74	3.63	2.12	12.58
	"	Med.	0.20	0.82	1.34	2.36	4.30	3.49	1.39	11.32
	"	High	0.40	0.97	1.72	2.69	3.40	2.08	1.40	8.92
	0.1	Low	0.10	0.71	1.31	2.02	3.06	2.94	1.35	12.96
	"	Med.	0.23	0.78	1.54	2.32	3.25	2.58	1.06	11.04
	"	High	0.37	1.00	1.70	2.70	2.73	2.35	1.70	13.14
	0.2	Low	0.29	0.82	1.62	2.44	2.54	2.09	1.08	12.58
	"	Med.	0.36	0.93	1.61	2.52	2.62	2.36	1.02	13.34
	"	High	0.37	0.93	1.59	2.52	2.29	2.28	1.12	13.34
	0.4	Low	0.48	0.97	1.73	2.70	2.19	2.35	0.80	12.92
	"	Med.	0.50	0.97	1.86	2.83	2.03	2.18	1.13	12.19
	"	High	0.58	1.05	1.93	2.98	1.64	1.90	0.90	12.80

though soil moisture regime is without effect at the highest salt level. The seemingly anomalous positions of the "low" and "medium" tension observations at "0" salt level cannot be adequately explained, and are probably merely fortuitous. It was even somewhat surprising that the "low" and "medium" tension curves showed a positive trend, since it has been observed (**5, 22**) that increasing concentrations of chlorides in the substrate effect

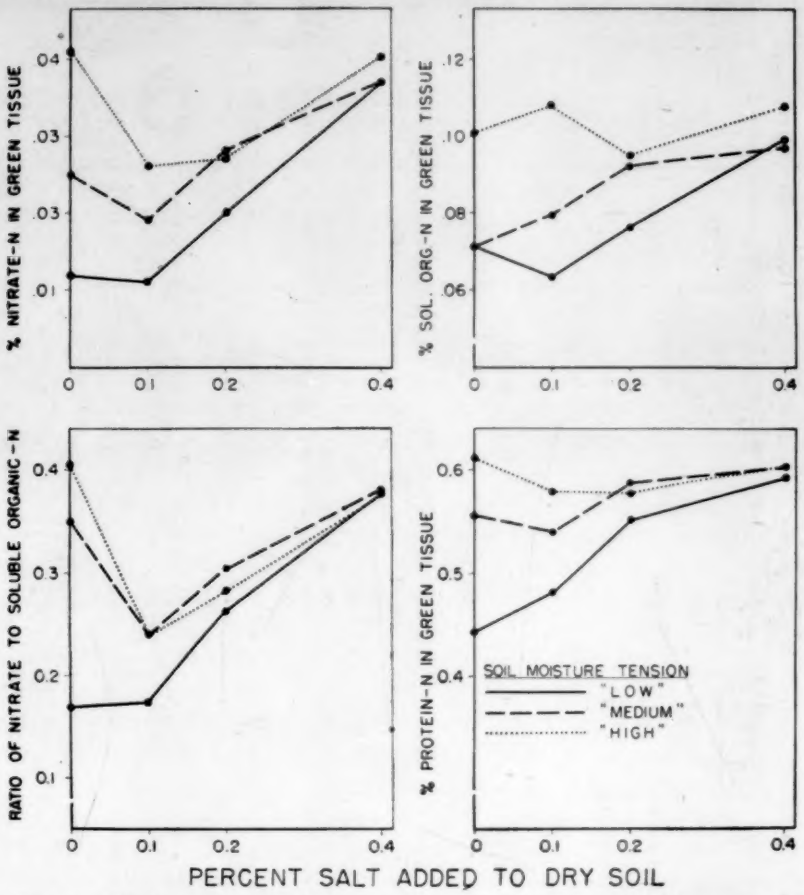

FIG. 6. Trends in the nitrogenous constituents in the leaves of the experimental plants.

either no change or an actual decrease in the percentage dry matter of plants.

Table II shows the percentage composition of various nitrogenous and carbohydrate constituents of the dry matter of the leaves and stems of these plants. Figure 6 shows the trends of the major nitrogen fractions found in the leaves on the green weight basis. Nitrate-N and soluble-organic-N found in the stems showed similar trends, whereas the "protein" content of stems showed practically no variation.

The level of nitrate nitrogen in the plants provides an index of the dif-

ferential between the rate of nitrogen absorption and the rate of nitrogen assimilation. This fraction is especially pertinent in this study in that the total nitrogen of plants from all treatments indicated that they should be characterized as "high nitrogen" plants. There is a decided trend for percentage of NO_3-N to increase with increased salt content of the substrate; and, also, for this percentage to increase with increase in degree of soil desiccation prior to irrigation. With few exceptions the differences between results from treatments having "0" and 0.1 per cent. added salt deviate from the general trend. This may be fortuitous, but LONG (**29**) has shown that when appreciable quantities of NaCl are present in the substrate, absorption of NO_3-N is reduced. The observed results suggest that the first increment of salt inhibited rate of absorption relatively more than rate of assimilation; but at high intensities of soil salinity, this intial effect on the differential between the two rates was moderated or even reversed.

The status of soluble organic nitrogen is usually an index of the level of nitrogen metabolism within plants. The trends of this component within the leaves are also shown in figure 6. The relative loci of these curves present a pattern approaching that observed for NO_3-N. At "low" and "medium" tensions, increase in soil salinity was associated with an increased content of soluble organic nitrogen in the leaves. The trend under "high" tension conditions tended to be the reverse. It is also evident (fig. 6) that there was a marked trend for the ratios of nitrate/soluble-organic-N to increase with increase in salt content of the soil, indicating that NO_3-N was absorbed faster than it was assimilated at the higher levels of soil salinity. In leaves from plants at higher moisture tensions, the first increment of added salt showed a reversal of this general trend.

Since the soluble organic nitrogen fraction is largely composed of amides, amino acids, and peptides—"building blocks" for proteins—it might be expected that the trends for the protein nitrogen content of these plants would line up correspondingly to those found for soluble organic nitrogen. This was found to be the case for the leaves but there was practically no variability in percentage protein in the stems. It appears that increases in both the osmotic pressure and the moisture tension of the soil moisture tended to increase the "protein" reserves in the leaves, but that when either variable was at its maximum stress the effect of the other was practically eliminated. Although the absolute range in these values for percentage of protein appears small, they are relatively large for leaves similar in age.

It is evident that the experimental treatments had a definite effect upon the status of nitrogen metabolism in these plants. But, what is more important, there was no evidence that nitrogen assimilation was inhibited to the extent of limiting growth. On the contrary, the highly important proteinaceous reserves tended to accumulate under the more intense conditions of stress.

One cannot fully interpret the nitrogen metabolism of plants without cognizance of the status of carbohydrate constituents. In contrast to the

similarity in relationships observed for the various nitrogenous fractions, it was found that various carbohydrate constituents showed certain differences in the relative trends incurred by the experimental conditions. The analytical results are presented in table II and partially in figure 7.

There were no significant differences in percentages of reducing sugars in the leaves, those from all treatments being low in this fraction. Bean

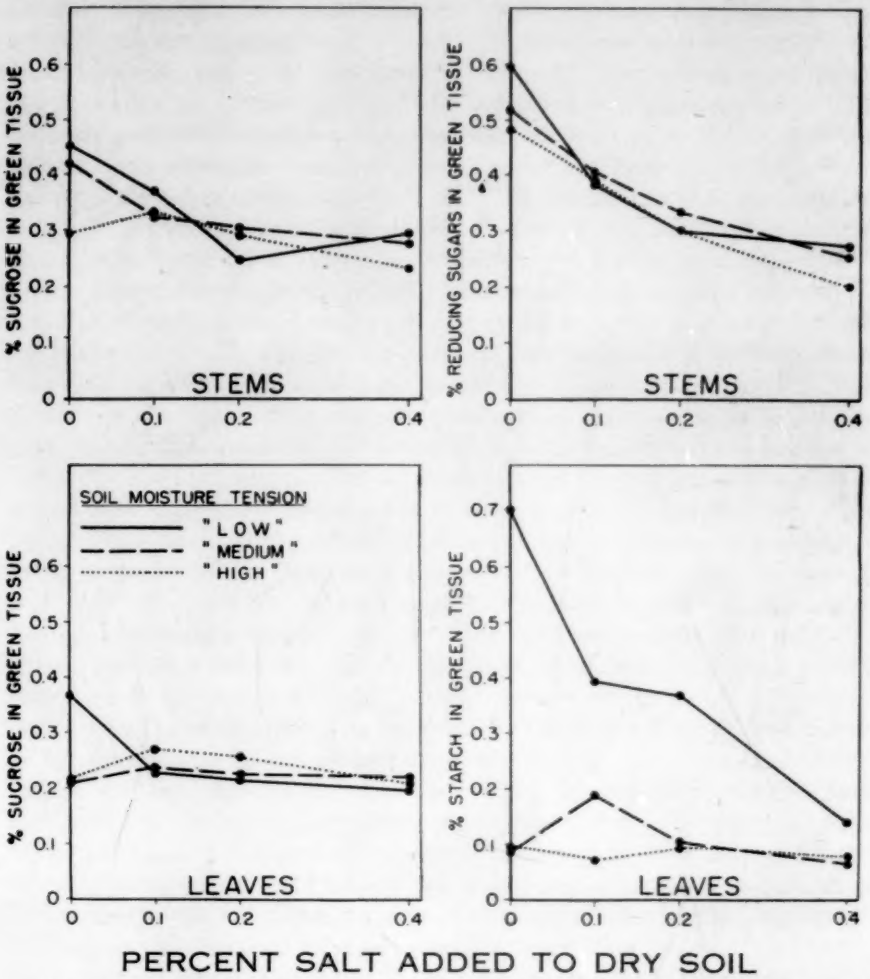

FIG. 7. Trends in the carbohydrate constituents of the experimental plants.

stems have a higher percentage of reducing sugars than the leaves, and soil salinity had a marked effect in decreasing the percentage of these sugars in the stems. Intensity of soil moisture tension appeared to have no effect on the level of these sugars. The parallelism noted for the effects of moisture tension and osmotic pressure of the soil moisture with respect to nitrogen metabolism is missing from the picture for reducing sugars. It is possible

that these trends reflect the specific effect of the chloride ion in lowering photosynthetic activity which has been noted for potatoes (**5, 41**) even though this effect was noted in the stems rather than the leaves. The results reported are at variance with those (**37, 38**) showing accumulations of reducing sugars in plants grown in artificial cultures of increasing salt concentration, and suggest that environmental conditions modify the effect of salinity on the status of reducing sugars in plants. The lowest percentages observed for this carbohydrate in no sense indicate a serious depletion with respect to affecting growth since vigorously vegetative bean stems may contain a considerably lower percentage of reducing sugars (**63**).

Variations in salt content of the soil had relatively little effect upon the percentage of nonreducing sugars in either leaves or stems, even though there was a slight tendency for percentage of sucrose to decrease with increase in soil salinity (fig. 7). Other studies on beans (**63**), sweet peas (**37**), and barley (**20**) grown on sand cultures presented evidence that saline substrates induce marked accumulation of nonreducing sugars. In fact, these sugars were comparatively abundant in plants of all treatments of the present study. In the cultures of the non-saline soil, the lower the soil moisture tension was maintained, the higher the percentage of "sucrose" in both stems and leaves. It appears that soil moisture tension and salt concentration of the soil solution had somewhat similar effects on the concentration of nonreducing sugars in these plants.

With respect to the starch reserves in the leaves of these plants, figure 7 shows that increasing either soil moisture tension or salt concentration of the soil had a marked effect. Variations in the starch content of the stems were negligible. At the higher levels of soil moisture tension, the starch content of the leaves was low and the salinity of the soil had little effect on the percentage of this constituent. This is in agreement with the results of another study with beans grown on saline soil (**64**) in which it was found that starch reserves were markedly depleted by soil desiccation even though the plants did not wilt, and the starch reserves were redeveloped within 24 hours after irrigating the desiccated soil. MAGNESS *et al.* (**35**) have found that soil moisture depletion induces a lowering of the starch reserves in apple trees. These observations are in accord with the findings of SPOEHR and MILNER (**52**) to the effect that moisture stresses within plants cause increased amylolytic activity and starch dissolution. Yet, it is of interest that the high percentage of starch in the leaves of the "0" salt, low-tension plants, as compared with the starch content of leaves from other treatments, was not compensated by any tendency for sugars to accumulate in the plants of these other treatments.

There is evidence (**47, 57**) that subjecting plants to conditions approaching drought causes a marked increase in the content of hemicelluloses in the tissues. These constituents have been considered important in the drought resistance of plants. Variation in the water relations of these bean plants induced by the experimental conditions had no definite effect on the status of hemi-reserves within the plants.

Discussion

Two of the main requirements for plant growth are the formation of protoplasm for new cells, and the hydration of that protoplasm together with vacuolation to bring about cell enlargement (**33**). The term "hydration" is here used in the same sense as implied by MACDOUGAL (**33**); *i.e.*, imbibition rather than formation of stoichiometric hydrates. It has been shown that the conditions of stress, which brought about marked growth reduction, actually caused an increase in the proportion of proteinaceous material in the tissues, especially in the leaves. This increase occurred even though the plants showing reduced rates of growth would have a comparatively small

TABLE III

OSMOTIC PRESSURE OF THE SOIL SOLUTION IN THE FOUR DIFFERENT SALT TREATMENTS AS INFLUENCED BY SOIL MOISTURE PERCENTAGES [FROM AYERS *et al.* (**1**)]

ADDED NaCl	DEPTH	CALCULATED* OSMOTIC PRESSURE† OF SOIL SOLUTION AT FOUR SOIL MOISTURE PERCENTAGES			
		7%	11%	15%	20%
%	*in.*	*atmos.*	*atmos.*	*atmos.*	*atmos.*
0.0	0–5	1.67	1.07	0.78	0.59
0.0	5–10	4.26	2.71	1.99	1.49
0.0	10–15	5.59	3.56	2.61	1.96
0.1	0–5	4.41	2.81	2.06	1.55
0.1	5–10	12.6	7.99	5.86	4.40
0.1	10–15	25.7	16.3	12.0	8.99
0.2	0–5	9.80	6.23	4.57	3.43
0.2	5–10	20.5	13.1	9.57	7.18
0.2	10–15	42.7	27.2	19.9	14.9
0.4	0–5	18.8	12.0	8.77	6.58
0.4	5–10	34.0	21.7	15.9	11.9
0.4	10–15	86.0	54.7	40.1	30.1

* Values calculated assuming only simple dilution or concentration.

† Osmotic pressures of soil solutions were determined on displaced solutions from soils adjusted to approximately 12 per cent. moisture.

proportion of young cells containing abundant protoplasm (**22, 37**); the type of cells capable of synthesizing proteins from nitrate. NIGHTINGALE and FARNHAM (**37**) drew the inference that this histological situation together with the *relatively* low percentage of protein nitrogen they observed, resulted in a condition of "protein deficiency" with respect to vegetative growth. It has been suggested (**32, 38**) that under conditions of stress inhibitory to growth, certain phases of protein synthesis may become limiting; *i.e.*, the total quantity of protein present gives no information on the quality of that protein or the prevalence of types specifically essential for growth. It was found, however (**64**), that beans almost completely resumed normal rate of growth within 24 hours after the alleviation of stresses such as those under discussion. This was especially shown in unpublished data on the daily rate of growth of expanding leaves and suggests that inability to form new protoplasm is not a primary growth restrictive under the conditions of the present study. The data indicate that there were adequate proteinace-

ous and other nitrogenous reserves for a higher rate of cell formation than was taking place in the stunted plants. Consequently, limited swelling of the protoplasmic proteins and decreased vacuolation of the protoplasm were undoubtedly main factors inhibiting growth whether the water stress was brought about by high soil moisture tension or by high osmotic pressure of the soil solution.

An indication of the range in osmotic pressure of the soil solution for various soil moisture percentages in the four different salt treatments is given in table III. These determinations were made on the same soil identically treated in a parallel experiment (**3**). These data together with those given in figure 1 show the extent of the decreases in free energy of the soil moisture which were induced by increasing osmotic pressure on the one hand, or increasing tension (fig. 1) on the other. Thus, under conditions of maintained low soil moisture tension (average soil moisture varied between 14 and 20 per cent.) the osmotic pressure in the surface horizon increased from about 0.8 to 9.0 atmospheres as the percentage of added NaCl increased from 0 to 0.4 per cent. Correspondingly, when the osmotic pressure was maintained at a low level ("0" added salt), depletion of the soil moisture caused an increase in the moisture tension from 0.3 to 12–15 atmospheres. In other words, decreasing the free energy of the soil moisture, whether by increasing moisture tension or by increasing osmotic pressure, would necessitate an increase in the diffusion pressure deficit within the plant in order to effect the "passive" entry of water into the roots. As a result, the free energy of the water in the plant tissues would also become lower. Decrease in the free energy of the dispersing medium is associated with a lowered degree of imbibition (swelling) of dispersed proteins (**49**). These facts support the conclusion that decreased imbibition by lyophilic colloids in developing cells is a factor limiting growth under the prevailing experimental conditions. Furthermore, a decrease in the free energy of the solution external to a cell would increase the osmotic work which would have to be done in bringing about vacuolation of the cytoplasm, thus inhibiting this phase of cell enlargement. THUT and LOOMIS (**54**) observed the diurnal growth of plants in relation to diurnal temperature, light, and humidity. They state that growth was checked by water deficits within the plant, and that such deficits were usually proportional to light intensity, temperature and air movement, and inversely proportional to relative humidity and the available soil moisture. The prevailing environmental conditions of the present experiment (*vide infra*) would certainly tend to accentuate the effect of soil moisture availability in growth.

EDLEFSEN (**14**) has suggested the elimination of most of the numerous and somewhat confusing terms dealing with water relations of plants. In their stead, he proposed evaluating the whole system in terms of free energy. This is a worthwhile motive. This concept has been especially valuable in studying the precise status of water in the substrate, but the complexities of plant processes which still reside in the realm of the unknown are not con-

ducive to thermodynamic evaluation in quantitative terms. The plant system is seldom in equilibrium.

In the light of the foregoing, growth response of the plants should be the net effect through time of water stress induced both by solutes and by mois-

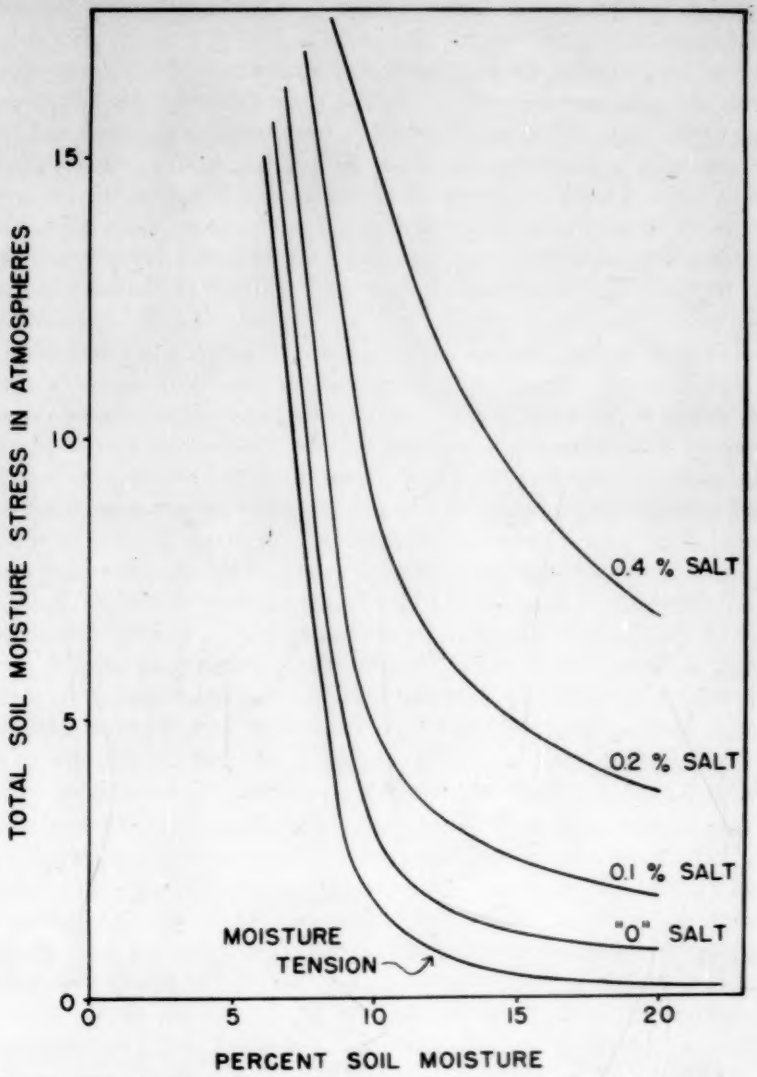

FIG. 8. The relation of moisture stress to moisture percentage at the different levels of soil salinity.

ture tension. Figure 8 indicates the minimum moisture stress to which the roots were subject at the various average soil moisture percentages. The curves for the various "salt levels" were derived by adding the osmotic pressure of the soil solution at the different average soil moisture percentages to

the respective values for the moisture tension. The observations for osmotic pressure employed were those found for the surface horizon of the soil—that stratum of the soil containing the least solutes. This was considered the most valid approach since it is shown in water cultures (**11, 29**) that the greater proportion of water taken up by roots is from the portion of the substrate having the lower osmotic pressure. The trend of moisture stresses found for the "0" salt treatments were not greatly different from that of the soil moisture tension, indicating that the increase in osmotic pressure of the soil solution due to soil moisture depletion was not a major factor in determining the moisture stress of this series. At the other extreme, represented by the "0.4 per cent.-salt" series, the osmotic pressure showed such

TABLE IV

THE ESTIMATED LOWER LIMITS OF THE AVERAGE MOISTURE PERCENTAGE AND THE INTEGRATED MOISTURE POTENTIAL FOR THE VARIOUS TREATMENTS

Moisture tension regime	Treatment			
	Percentage added salt			
	"0"	0.1	0.2	0.4
Lower limits of average soil moisture percentage				
	%	%	%	%
Low	14	14	14	14
Medium	10	10	11	11
High	6	7	8	9
Integrated moisture stress* between the above respective lower limits and 20 per cent. moisture				
	atmos.	*atmos.*	*atmos.*	*atmos.*
Low	1.1	2.2	4.4	8.2
Medium	1.5	2.8	5.1	9.4
High	3.9	4.6	6.7	10.5

* Arrived at geometrically.

a marked increase with soil moisture depletion that the soil moisture tension was a negligible consideration in the trend of the total moisture stress for this series.

It is difficult to arrive at the integrated moisture stress acting on the absorbing roots between an irrigation and the designated degree of soil moisture depletion. The prescribed degree of water removal from the soil for a given treatment varied from only one day to 10 days (fig. 2). A rough index of the effective moisture stress over an irrigation period may be gained by integrating the respective moisture stress curves within specified limits. These limits of the average soil moisture percentage were available from the data which gave rise to figure 2. The maximum average moisture percentage in each case was 20. The lower limits are given in table IV as well as the

integrated moisture stresses. The average green weight per plant is plotted against these respective moisture stresses in figure 9. The closeness with which these paired observations follow a general trend probably involves a modicum of fortuity. It is obvious that these integrated moisture stresses are crude approximations since no information is available as to the status of water relations at the surface of the roots as contrasted with the average

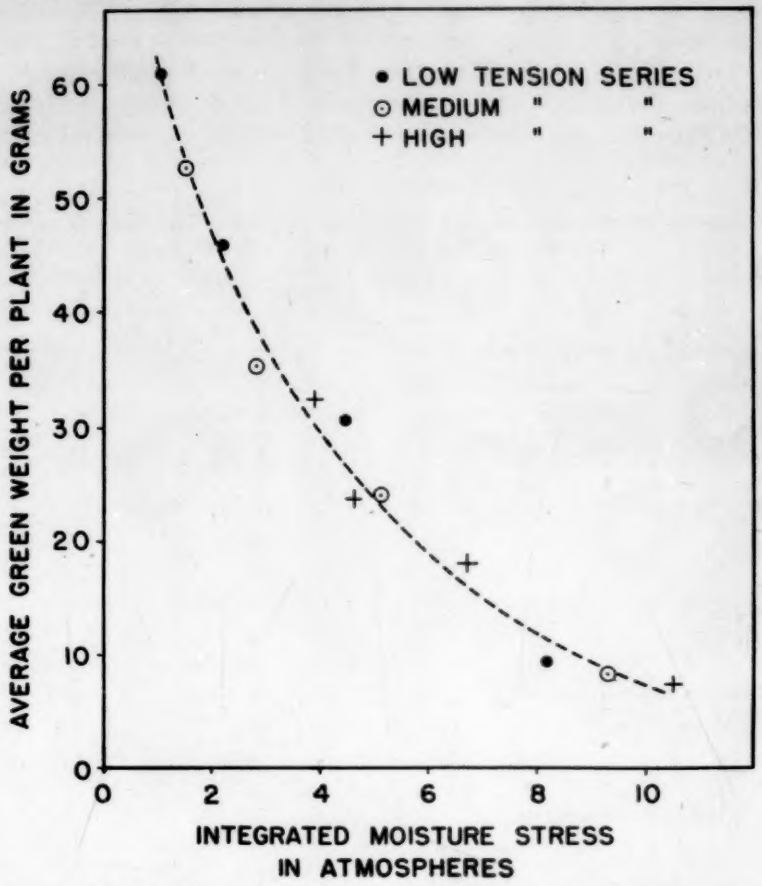

FIG. 9. The relation of plant growth to the "integrated moisture stress."

for the whole soil mass. Nevertheless, a plant has no alternative to precisely integrating all the stresses placed upon it within its inherent limitations. Relative intensities of stress in water supply may be reasonably expected to induce relative inhibitions in growth response. Consequently, the data on growth become approximate evaluations of the relative reliability of the integrated moisture stresses.

The discussion so far has been in support of the view that varying degrees of water availability were a *major factor* in bringing about the observed responses. In continued support of this conclusion, the observations on per-

centage of starch confirm those of another study (**64**) with regard to the effect of either increasing soil moisture tension or of increasing osmotic pressure of the soil solution in bringing about a depletion in starch reserves within the plant. This may have been due in part to starch hydrolysis through increased amylolytic activity (**52**), but there were no accumulations of the hydrolytic products of starch to compensate for its disappearance. The findings of SCHNEIDER and CHILDERS (**50**), that moisture stress brings about a decrease in apparent photosynthesis and an increase in respiration of apple leaves, are pertinent. The data showed a decreased degree of nitrate reduction under most of the conditions of stress. Nitrogen assimilation is ordinarily a main channel of carbohydrate utilization, and a low level of nitrogen metabolism usually results in carbohydrate accumulation. Since the low rate of growth under conditions of stress would require correspondingly low quantities of synthesized nitrogenous constituents, carbohydrates would accumulate if photosynthetic activity had been maintained at the normal rate. Yet, there was an actual depletion in carbohydrate reserves accompanying intensified soil moisture stresses. It should be noted that during the last few days prior to harvest the temperature of the greenhouse reached a maximum of 93–96° F. and the relative humidity reached a minimum of 25–35 per cent. Such high temperatures are probably superoptimal for photosynthesis in beans. This possibility together with the relatively high rate of transpiration which would be induced by such an aerial environment and which would develop a diffusion pressure deficit within the leaves sufficiently high to inhibit photosynthesis, undoubtedly account for low reserves of photosynthetic products.

The authors believe that factors other than varying degrees of water availability were involved in bringing about differences in the biochemical status of these plants. The trends for reducing sugars in the stems did not appear to be related to moisture tension even though salt concentration in the soil had a marked effect on these sugars. Unpublished observations from investigations reported by MAGISTAD *et al.* (**34**) indicate that bean plants grown on high concentrations of chloride salts tend to develop etiolation in contrast with the deep green color of the leaves of plants grown on cultures with a high concentration of sulphate. In view of this and other studies (**4, 5**) it appears that an excessive supply of chloride salts is inhibitory to an accumulation of photosynthetic products. Although the indications of such a condition were evident in these plants, it is doubtful if this specific effect of the chloride ion had been a significant factor in the accumulation of dry matter up to the stage of growth considered. In another study (**21**), bean plants also harvested at the stage of incipient flowering showed as great or even greater reductions in growth in the presence of excessive concentrations of sodium sulphate as of sodium chloride at isosmotic pressures.

Some of the foregoing conclusions are not in harmony with observations of other investigators working on allied problems. The fact that an increase in soil moisture tension from 0.2 to 15 atmospheres would necessitate

an associated increase in diffusion pressure deficit in the absorbing cells of the roots to initiate the passive entry of water is at variance with the evidence that all soil moisture between field capacity and the wilting percentage [15–18 atmospheres of tension (**13, 44, 53**)] is equally available to plants. Furthermore, some investigators regard the interpretation of the effect of saline substrates on plant growth in terms of osmotic forces as fallacious (**12**). In other words, the effect of the salt in the soil solution is sometimes regarded as chemical (toxicity) rather than physical. From this standpoint, the effect of depletion of soil moisture on increasing the salt concentration of the soil solution in the "0" salt series (table III) suggests the possibility that salt toxicity rather than the increase in soil moisture tension was the active factor in depressing growth in this series. Certainly, the increase in osmotic pressure associated with decrease in soil moisture cannot be disregarded whatever the basis for interpretation. It is evident that if soil moisture is equally available to plants over the moisture tension range of 0–15 atmospheres, then water should also be equally available to plants even though the osmotic pressure of the culture solution was increased from 0.5 to 15 atmospheres by a non-toxic solute. VEIHMEYER and HENDRICKSON (**60**) support this contention with the observation that sunflower plants grown in sand cultures and transferred to sucrose solutions were able to withstand concentrations corresponding to 16 and 20 atmospheres before wilting. Neutral salts affect water absorption and plant growth when the osmotic pressure is only 2 to 4 atmospheres (**11, 22, 23, 29, 34, 37, 48**). However, results of other investigators are not in agreement with those of VEIHMEYER and HENDRICKSON (**60**) as to the magnitude of osmotic pressure of a sucrose solution that plants will withstand without an appreciable effect on water absorption. URSPRUNG and BLUM (**56**), ROSENE (**48**), and LONG (**29**) observed a high degree of inhibition in water absorption by roots when the concentration of sucrose in the substrate was only 4–5 atmospheres. The two latter investigators noted the same effect on water absorption whether the osmotic pressure of the substrate was built up with sucrose or a neutral salt (KNO_3 or NaCl). The preponderance of data indicates that neutral solutes affect water availability to plants by means of osmotic forces even when the osmotic pressure is only a few atmospheres.

The results of some investigators do not support the view that all soil moisture above the wilting percentage is equally available to plants. KRAMER (**28**) found that exudation from the stumps of several species of plants in several different soils ceased when about 45 per cent. of the moisture available to the intact plants was still present. The results suggested that the soil moisture content limiting exudation is as characteristic of a soil as are the wilting percentage and the moisture equivalent, and that active absorption does not take place against a diffusion pressure deficit greater than one or two atmospheres. KRAMER concluded that his findings did not support the theory of equality in availability of soil moisture present in excess of the wilting percentage. Numerous other investigators have found modifications

in plant response due to decreasing soil moisture content even though the plants did not wilt (**9, 15, 18, 35, 50, 64**).

The hyperbolic nature of the moisture sorption curve given in figure 1 provides information relevant to the finding that for all practical purposes variations in soil moisture content above the wilting percentage are frequently found to be without influence on plant growth. The general trend of this curve is typical (**61, 62**), but its exact locus will shift with different soils. The nature of this curve shows that most of the available water in the soil is removed even when only one atmosphere of tension has developed. Removal of a small quantity of available water just above the wilting percentage involves an enormous change in moisture tension. Such a rapid rate of change in tension with moisture percentage makes it practically impossible to evaluate a given soil moisture tension of 3 to 15 atmospheres in terms of plant growth. The effective tension at the absorbing surface of the roots is at a given value in this range for only a brief interval, and it is impossible to maintain a given tension in this range (**25, 44**). Consequently, rate of movement of water through the soil to the root membrane probably becomes more of a limiting factor in moisture availability than average moisture percentage in the root zone (**2**).

MOORE (**36**) has shown that unsaturated flow of moisture in soils is very low at and below the moisture equivalent. This means that there may be a high gradient in moisture stress between the absorbing surface of the root and the soil particles a few millimeters away. VEIHMEYER and his collaborators (*loc. cit.*) have stressed that the range of "available" moisture is meaningless if the roots have not thoroughly permeated the soil mass. In this connection, it is noteworthy that the moisture tension at the wilting percentage may vary from 10 to 24 atmospheres (**6, 60**) or even more (**46**). It is suggested that sunflower plants do not show such an extreme range in their critical diffusion pressure deficit, but that this wide range in the free energy of the soil moisture at wilting percentage reflects inherent inaccuracies in the method. Moreover, it has been shown (**18, 19, 59**) that the wilting percentage is actually a narrow range in soil moisture percentage through which the plant shows increasingly severe wilting. The moisture sorption curve in figure 1 shows why even a narrow range in soil moisture percentage would be associated with a wide range in moisture tension near the wilting percentage.

Summary

Dwarf Red Kidney beans were grown to incipient flowering in 10-gallon containers filled with a loam. These soils contained 0, 0.1, 0.2 and 0.4 per cent. of added sodium chloride on the dry soil basis. The 36 cultures were divided into three moisture tension series. Water was added when the soil moisture tension at the 4-inch depth had reached 250 cm. of water and 750 cm. of water for the first two series, respectively. Water was added to the third series when plants were wilted by mid-morning, corresponding to tension greatly exceeding 800 cm. of water.

Plant growth was inhibited as the soil moisture tension at time of irrigation increased, even though in some of the treatments the soil moisture was always above the wilting range.

Progressive additions of sodium chloride to the soil caused progressive decreases in growth and yield of beans.

Increasing soil moisture tension or salt concentration tended to cause an increase in the percentage of nitrate nitrogen in the plants, and to have a similar effect, though less pronounced, on the percentage of soluble organic nitrogen. Consequently, increasing intensity of either type of stress tended to cause an increase in nitrate/soluble-organic-N ratio. The percentage of protein in the leaves increased with increased intensity of either type of stress.

Soil moisture tension had no effect on percentage of reducing sugars whereas increasing salt concentration was associated with a definite decrease in percentage of these sugars in the stems. The experimental treatments had little effect on percentage of nonreducing sugars or percentage of hemireserves. Increasing either soil moisture tension or salt concentration caused a very marked decrease in percentage of starch in the leaves.

The relative effects of salt concentration and soil moisture tension on water availability to these plants is discussed. It is concluded that growth reductions were to a large extent brought about by reduced hydration of protoplasmic proteins, whether water stress was due to osmotic forces or to moisture tension, since adequate percentages of proteinaceous constituents were present for higher rates of meristematic activity.

It is suggested that factors other than varying degrees of water availability were operative in conditioning these plants. The data indicate that excessive concentrations of the chloride ion *per se* affect carbohydrate metabolism, possibly through reduced photosynthetic activity.

It is concluded that the hyperbolic nature of the relationship between soil moisture percentage and moisture tension accounts for the frequent finding that for all practical purposes plants may not show changes in growth response while reducing the moisture percentage of soil from field capacity to nearly the wilting percentage.

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CHANGES IN ELECTRICAL POLARITY IN THE AVENA COLEOPTILE AS AN ANTECEDENT TO HORMONE ACTION IN GEOTROPIC RESPONSE

A. R. SCHRANK

(WITH ONE FIGURE)

Introduction

The coleoptile of *Avena sativa* has been used extensively in the study of geotropic curvature (**1, 10**). When the coleoptile is placed in the horizontal position, it responds to the force of gravity as the stimulus by curving upward, by accumulating a greater quantity of growth hormones in the lower than remains in the upper half, and by establishing a transverse electrical polarity oriented so that the under side becomes electropositive to the upper side in the external circuit. Although there may be other concurrent responses, this paper is concerned only with these listed. It is generally accepted that the unequal hormone distribution is an essential precursor for the upward bending (**1, 10**), but these hormones must be *directed* to the under side by some orienting *force* (*polarity*). It has been suggested (**1, 10**), though it has not been proved, that the transverse electrical polarity might be the required orienting force. If the electrical polarity is to fulfill this requirement, then its establishment must chronologically precede the unequal growth hormone distribution. Consequently, an analysis of the experimental data on this point is in order.

Experimental

The curve in figure 1A is an average of 6 experiments showing the electrical responses of Avena coleoptiles when they are placed in the horizontal position. These electrical measurements were taken at points 2 millimeters below the apex. The contact on the upper side was connected to ground, and the contact on the under side was used as the variable. The details of the experimental procedure whereby these data were obtained have been reported elsewhere (**6, 7**). These experiments were performed on coleoptiles that were 32 ($\pm$) millimeters long at an average temperature of 21.8 ($\pm$ 0.25) degrees Centigrade. Since the curve in figure 1A shows a transverse electrical polarity of 1.5 millivolts after 1 minute in the horizontal position, it reveals the fact that the appearance of this polarity can be detected within one minute or less. It is very probable that this electrical polarity could be demonstrated much sooner with an instrument of shorter period and greater sensitivity. The data in figure 1A are in agreement with the results of BRAUNER (**2**) and WILKS and LUND (**11**).

Data which have been published by DOLK (**3**) can conveniently be used for demonstrating the time required for an unequal distribution (in the upper and lower halves of the coleoptile) of growth hormones to occur as

the result of stimulation by gravity. DOLK placed coleoptiles in the horizontal position for a minimum of eleven minutes. At the end of this time the plants were decapitated and the tips placed on an apparatus on agar so that the upper and lower halves of the tips were separated by the edge of a razor blade. After leaving for a certain time on agar, the amount of growth substance was determined in the usual way (**1, 10**).

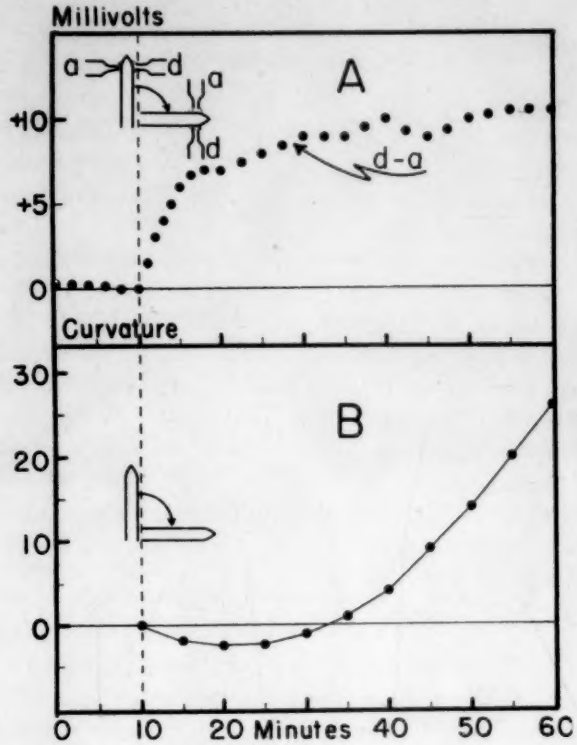

FIG. 1. A. Changes in the transverse E.M.F.'s in Avena coleoptiles when changed from vertical to horizontal positions. Contacts 2 mm. below apex. Curve represents average of 6 experiments.

B. Upward curvature of Avena coleoptiles in horizontal position measured in ocular scale division of 18 per millimeter. Average of 10 experiments.

The first line in DOLK's (**3**) table IV indicates that the test blocks from the under side induced a curvature of 1 degree more than the test blocks from the upper side. A consideration of the experimental procedure used and a comparison of results obtained from identical experiments as shown in this table make it extremely doubtful whether a difference in curvature of 1 degree is significant.

The time that elapses before upward curvature begins can accurately be determined. Figure 1B shows the average geotropic curvature of 10 coleoptiles, each approximately 30 millimeters long. The curvature was measured by and is plotted in scale divisions (18 per millimeter) of an ocular microme-

ter. These experiments were performed at an average temperature of 25.2 (± 0.25) degrees Centigrade. This curve demonstrates that upward curvature can be detected after 22 minutes in the horizontal position. The initial sagging of the curve in figure 1B is probably due to the weight of the coleoptiles.

Discussion

As far as experimental measurements are concerned, as demonstrated by the data cited in this paper, the following sequence of responses of the Avena coleoptile to gravity has been conclusively settled: (1) Establishment of a transverse electrical polarity. (2) Unequal distribution of the growth hormones (**3**). (3) Upward curvature. However, the fact that a difference in hormone distribution has not been measured prior to eleven minutes in the horizontal position does not necessarily mean that a significant difference does not occur sooner. For this reason some relevant theoretical aspects should also be considered.

The growth hormones could not be transported from the upper to the lower side by simple diffusion; because, first of all, the required concentration gradient of growth hormones does not exist. Secondly, it has been shown that the velocity of hormone transportation is almost completely independent of temperature (**8, 9**). If diffusion were involved, this would not be true. The velocity of longitudinal hormone transport has been measured experimentally in the Avena coleoptile and is conceded to be about 10 millimeters per hour (**5, 8, 10**). If the minmum distance for the hormone to be transported in going from the upper to the lower side is assumed to be one millimeter,[1] then 6 minutes would be required for the hormones to reach the under side. Even if it were assumed that the growth hormones are transported a distance of 1 millimeter from the upper to the under side directly by protoplasmic streaming without hindrance by the cell walls, and the velocity of protoplasmic streaming is taken to be 10 microns per second (**4**), then 1.6 minutes would elapse before the hormones could reach the under side. From this study it becomes clear that the transverse electrical polarity is actually established in the Avena coleoptile *before* a difference in hormone concentration is considered possible. If a transverse electrical polarity is required for the lateral transport of growth hormones, then it obviously must be established previous to the unequal hormone distribution. The fact that the transverse electrical polarity is established prior to the unequal hormone distribution does not necessarily mean that it is the orienting force or polarity ["transverse polarization of the cells," *op. cit.* (**10**), p. 157] which is essential for lateral hormone transport. It means only that

[1] This assumption is well within reason because the transport of growth hormones from one side of the coleoptile to the other probably occurs in a basipetal diagonal direction rather than perpendicular to the longitudinal axis. The fact that the zone of curvature proceeds from the apex towards the base indicates that this concept of diagonal transport is correct.

the transverse electrical polarity fulfills the prime requirement of being established in the correct sequence.

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RENEWAL OF GROWTH BY GUAYULE TRANSPLANTS[1]

T. C. Broyer

(WITH TWO FIGURES)

Guayule seedlings were received from the federal nursery of the Emergency Guayule Project at various times of the year for use in a general study of the growth of this species under greenhouse conditions (1). Stock removed from the nursery at different times of the year showed markedly different powers of regeneration when transplanted to soil in the greenhouse. The best growth was obtained from stock transplanted from the nursery during the winter. As the season advanced from winter toward summer, a progressive decrease was observed in the ability of nursery stock to resume growth in certain soils. The resumption of growth of stock received from the nursery in June was very poor. A comparison of regeneration in soil contrasted with that in water culture is of particular interest.

Seedlings were shoot pruned in the nursery beds at successive intervals from late December, 1942, to early July, 1943. Shortly thereafter, as is usual in preparing for transplantation, these seedlings were removed from the soil after partial root pruning. The effects of pruning were accentuated at the time of experimentation by further abscission to reduce the transpiration surface to a minimum and by root trimming to expose fresh root surfaces for more ready entry of water. Seedlings were transplanted as soon as convenient after shipment—usually 1 to 2 weeks after removal from the nursery soils. Whenever possible, storage temperatures were maintained at about 15° C. Experiments were performed in vessels containing nutrient or soil media.

Successive lots of plants transplanted into the same type of soil renewed growth progressively more slowly, and the percentage regeneration decreased. The same phenomenon obtained when plants were placed directly in nutrient solutions—however, to a much smaller degree. In aqueous solutions the regeneration at all times was more rapid and a larger percentage of renewed growth was obtained. Within relatively wide time limits the absolute regeneration observable is merely a measure of rate. The rate of renewal of growth is possibly related, in one way or another, to the time of shoot and root pruning in relation to the phase of the plant's periodic growth cycle.

Conceivably, the availability of moisture in soils is a prime factor governing the regeneration relative to nutrient solution cultures. To test this point, when resumption of growth in soils was otherwise poor, successively increasing average moisture percentages were maintained in lots of Fresno fine sandy loam and Danville clay loam (from Richmond). The rate of

[1] A report of observations reported at the Winter Research Conference of the Guayule Emergency Rubber Project, Salinas, California, February 5, 1944.

regeneration was essentially the same throughout the series. Scoring of the root stump did not influence the results. Further, pre-treatment of plants in these same soils, by subjecting the roots to maintained low temperatures (5° to 15° C.), did not affect their inability to renew growth when subsequently transferred to more favorable temperatures.

Another approach was made to the problem of regeneration in the summer season. Nursery plants were placed in a large tank containing Fresno fine sandy loam immediately and after intervals of 2 hours, 8 hours, 2 days, 4 days, and 1 week of presoaking of the roots in water. At this time, in

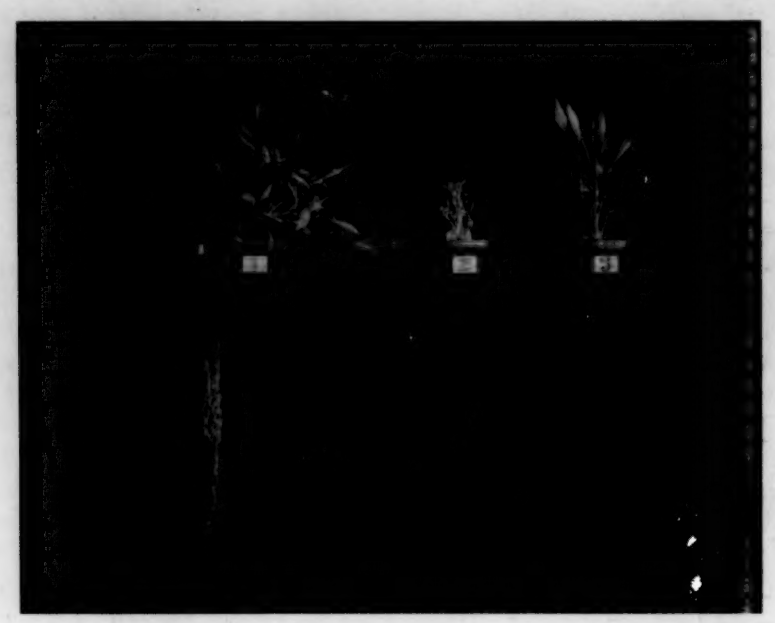

FIG. 1. Growth of plants transplanted into nutrient solution and soil: Plant *1* was transplanted into Fresno fine sandy loam at 12 per cent. moisture (wet basis) and at the end of two weeks was transferred to aerated standard nutrient solution for 5 weeks; plant *2* was left in the Fresno fine sandy loam for 7 weeks; and plant *3* was transplanted to the soil for 2 weeks, transferred to aerated standard nutrient solution for 1 week, and then returned to the soil for another 4 weeks. All plants were transplanted to the soil on June 2 and photographed July 27, 1943.

the course of 2 to 3 weeks only about 5 per cent. of the plants regenerated in the soil, with no relation to the control treatment or to the interval of root presoaking. Yet similar plants whose roots were held in water, in dilute calcium sulphate or in a standard nutrient solution, with or without forced aeration, produced a high percentage of regeneration (root and shoot development was initiated in about 1 week). Further, the dormant plants from the soil, when subsequently transferred to an aerated standard nitrate nutrient solution very soon resumed root and shoot development and continued active growth. If plants subjected to the soil treatment,

followed by the nutrient solution treatment until root and shoot development was resumed, were then returned to the soil, continued growth was markedly retarded. Figure 1 shows representative plants from these treatments. Although no conclusion could be drawn from these observations, several suggestions may be offered. Aeration in the soil may be inadequate for plants to renew growth at the particular season of the year. Toxic substances or inhibitors of regeneration may be found in the soil, associated with a seasonal effect on the growth rate of certain soil microflora. Similar substances may be produced in the field stock, progressively higher in concentration as the season, size of plant, or physiological cycle of growth advances. (Note that in midwinter with nursery plants received in January, the regeneration in this soil was nearly complete.) These substances might be slowly lost to the rooting medium; in the solution they might be diluted enough to be relatively ineffective, or in the solution or within the plant itself they might be oxidized by higher aeration, whereas in the soil they might be retained at or near the root surface by the soil colloids and possibly at a lower oxidative level.

To test these possibilities, further soil cultures were arranged. Plants in soil, held within a favorable moisture range and aerated (by means of a carbon aerator placed along the bottom of the tank), were comparable with controls without aeration. Regeneration in sterilized soil was equal to, or perhaps slower than, that from check treatments. The resumption of growth was neither markedly increased nor inhibited by presoaking in solutions of β-indole acetic acid (50 p.p.m.) or of other growth factors (thiamin, nicotinic acid, pyridoxin, ascorbic acid, glycine), or by application of such solutions to soil. Addition of the standard nutrient solution to the soil had no beneficial influence. Alternate heavy wetting and relative drying proved less satisfactory than a maintaned favorable moisture condition. A 1:1 soil-water extract of Fresno fine sandy loam in which guayule nursery plants had failed to grow actively (fig. 1) was prepared. With this 1:1 extract as a nutrient solution, growth was compared with that of plants similarly placed in a synthetic standard nitrate nutrient solution. Both aerated and unaerated cultures were compared for the two solutions. As figure 2 shows, development in the soil extract was less favorable than in the control nutrient. This was particularly true where aeration was not adequate.

SMITH (**5**) has recently published his results of an independent study carried on over the same interval of time, in which he presents detailed observations on the inhibition of growth of transplants as affected by topping and defoliation. He relates the inhibition to an inhibiting agent coming from the leaves and terminal buds. He states ". . . topping was apparently instrumental chiefly in removing the inhibiting organs." When "growth was resumed within a few days time" it was considered that "the residual agent was inactivated or 'used up.'" SMITH (**4**) has reported a favorable growth response by nursery stock whose roots were soaked in solutions of indole butyric acid (1 p.p.m.) prior to transplanting. These

results, suggesting, first, an inhibition of regeneration by factors in the shoots of transplants and, second, a beneficial effect of pretreatment by indole butyric acid are difficult to explain, as is the general mechanism of auxin function [compare (**5**), p. 335].

TINGEY, FOOTE, SAMPSON, and COWLEY (**6**), in a study of the effects of top and root clipping as related to time of planting, found that less pruning gave better regeneration on transplanting. ERICKSON (**2**) found that preparation of nursery stock for transplanting by hardening, either through chilling or reducing the moisture percentage of the plants, was beneficial for

FIG. 2. Growth of plants in aerated and unaerated solutions of standard nutrient and soil extract: Plant *1* was transplanted into aerated, standard nutrient solution; plant *2* into unaerated, standard nutrient solution; plant *3* into an aerated 1:1 water extract of cropped Fresno fine sandy loam in which the palnts used had remained relatively dormant for 1 month; and plant *4* was in unaerated 1:1 water extract of cropped Fresno soil. Plants *3* and *4* were not materially benefited by subsequent additions of standard nutrient solution to the soil extracts. Plants were transplanted July 1 and photographed July 27, 1943 (4 weeks' growth).

regeneration. HILGEMAN (**3**) reported that in Arizona the percentage survival of transplants increased from June to January. In the course of the year, apart from specialized treatments applied to nursery stock to maintain a high percentage of regeneration on transplanting, there appears to be an annual cycle imposed upon this species in its ability to regenerate on transplanting in soil.

In the present study the nursery stock was similarly pruned within an experiment and as the season advanced. The differences here, therefore,

are not primarily related to the topping or defoliation conditions but were strikingly affected by environmental situations arising from the differential root culture; *i.e.*, soil and solution (or water) media. It is difficult to visualize the soils employed as poorly aerated. Regeneration actively occurred in unaerated water even in the summer season when growth renewal was poorest in soils. Although the difference in regeneration may be related to the presence of growth inhibitors it seems unlikely that they are reduced in effective concentration directly within the plant in these experiments.

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A CRITICAL EVALUATION OF THE TERMINOLOGY OF DIFFUSION PHENOMENA[1]

BERNARD S. MEYER

Diffusion phenomena are involved, directly or indirectly, in practically all fundamental plant processes, playing particularly important rôles in the movement of substances into and out of plants and in the translocation of substances within the plant. In recent years the results of a number of different lines of investigation, which need not be cited here in detail, have increasingly indicated that energetically much more complex mechanisms than diffusion are involved in the transfer of at least certain kinds of solute particles, and perhaps also water molecules, from the environment into the plant, from the plant into its environment, and from cell to cell within the plant. In spite of such more complex mechanisms which are superimposed on the kinetically and energetically simpler process of diffusion, this latter process does account principally or entirely for certain of the movements of materials which occur within a plant or between a plant and its environment. Furthermore, diffusion almost invariably accompanies, as a subsidiary or underlying mechanism, those processes in which the predominant mechanism is thermodynamically more complex.

The now generally recognized fact that diffusion is only the simplest of the mechanisms which play a part in the movement of substances into, out of, and within plants should not disincline us from attaining as clear an insight as possible into the dynamics of this process. A lucid picture of the kinetics of diffusion phenomena as they occur in relation to plants is necessary as a foundation for an understanding of other more complicated processes involved in the movement of materials in plants. In this paper little attempt is made to explain or conceptualize even the relatively simple phenomena of diffusion, but only to evaluate the terminology used in descriptions of such phenomena, and to make certain recommendations regarding preferences in such terminology. Any clarification or simplification in terminology which can be accomplished should, however, make possible a more effectual presentation of the concepts of diffusion.

The fact that the terminology of diffusion phenomena is far from being stabilized is the principal justification for the writing of this paper. Physi-

[1] A report of the Physical Methods Committee of the American Society of Plant Physiologists. The membership of the committee is as follows: S. T. DEXTER, K. C. HAMNER, B. S. MEYER, GORDON MARSH, and R. B. WITHROW, Chairman. This manuscript has been critically read by all members of this Committee and also by DR. O. F. CURTIS of Cornell University, DR. C. A. SHULL of the University of Chicago, DR. H. C. SAMPSON of the Ohio State University, DR. D. B. ANDERSON of North Carolina State College, DR. A. S. CRAFTS of the University of California, DR. P. J. KRAMER of Duke University, DR. R. O. FREELAND of Northwestern University, DR. H. T. SCOFIELD of the Ohio State University, and DR. C. A. SWANSON of the Ohio State University. All of these persons have made valuable suggestions and criticisms which are hereby gratefully acknowledged. The author alone, however, is responsible for the final form in which all statements are made.

cists and chemists seem to have been no more consistent in their use of many of the terms descriptive of these phenomena than biologists. Hence no solution of this problem can be attained by the convenient expedient of borrowing definitions from the physical sciences.

Absolutely rigid definitions are usually impracticable in any science, and even if once formulated eventually may prove to be untenable. A rigid stabilization of terminology is no more possible than a rigid stabilization of language or of concept. Uncritical adherence to precise definitions is more likely to block progress in thinking than to advance it. The best we can hope to do is to seek for the most useful working concepts possible in the light of current understanding and to embody them in statements or definitions which admittedly cannot be too precise. A reasonable consistency in the use of a given term for a given concept would, however, seem to be a legitimate desideratum in any branch of science.

Diffusion

The basic concept of all diffusion phenomena is that of the dynamics of the diffusion process itself. This process is most easily visualized as it occurs in gases. For example, in an actively photosynthesizing leaf with open stomates, the partial pressure of carbon dioxide is less in the intercellular spaces than in the surrounding atmosphere. As a result of their random kinetic activity certain of the atmospheric carbon dioxide molecules pass through the stomates into the intercellular spaces. Simultaneously, and also as a result of their kinetic activity, certain other carbon dioxide molecules pass from the intercellular spaces through the stomates into the outside atmosphere. Because of the greater partial pressure of carbon dioxide in the atmosphere than in the intercellular spaces the number of molecules passing per unit of time in the inward direction will exceed the number passing in an outward direction. Such a net movement of the molecules of a given substance in one direction is termed *diffusion*. Under conditions favorable for photosynthesis continued solution of carbon dioxide in the moist walls of the mesophyll cells occurs and inward diffusion of this gas may continue uninterruptedly for hours at a time.

If, however, solution of carbon dioxide molecules in the water-saturated cell walls ceases, the partial pressure of the carbon dioxide in the intercellular spaces soon becomes equal to that in the outside atmosphere. Movement of the molecules of carbon dioxide continues under these conditions, but the number passing through the stomates in one direction per unit time interval will be equal to the number passing in the opposite direction. When this condition of dynamic equilibrium has been attained diffusion is no longer occurring.

The *direction* of the diffusion of a gas is invariably from a region or regions of its greater partial pressure to a region or regions of its lesser partial pressure. The partial pressure of a gas is sometimes referred to as its *diffusion pressure* (see later); in this context this term can be appro-

priately substituted for partial pressure when discussing diffusion phenomena. The *rate* of diffusion, on the other hand, is influenced by a number of factors including the steepness of the diffusion pressure gradient (see later), temperature, the volume and mass of the individual diffusing particles, and the properties of the medium through which diffusion takes place.

Not only do the molecules of gases diffuse, but so also do the molecules of liquids and solids, the molecules and ions of solutes, and the micelles of colloidal sols. However, because of intermolecular and interionic attractions and repulsions, association of molecules, and perhaps other factors, the diffusion of liquids and solutes is not strictly comparable in kinetic simplicity with the diffusion of gases. In spite of these complications there is a close resemblance between the principles which hold for the diffusion of gases and those which seem valid for the diffusion of liquids and solutes.

A distinctive feature of diffusion is that the molecule or other particulate form of matter is the unit of movement and the process is distinguished in this way from mass movements such, for example, as convection currents or protoplasmic streaming in which the unit of movement is a large assemblage of molecules or particles.

The term diffusion is sometimes employed to refer to random movement of molecules as a result of their intrinsic energy ("molecular movement") a usage which identifies diffusion with kinetic activity and makes the former a superfluous word. Another use of the term diffusion is exemplified in such statements as "diffusion of carbon dioxide occurs more rapidly in an inward than in an outward direction." If diffusion is used in this sense some such term as "net diffusion" is needed to designate the phenomenon called diffusion in the preceding discussion. The simpler usage presented in the first paragraph of this section would seem to be preferable and furthermore is consistent with the accepted meanings for osmosis and imbibition, terms for special cases of diffusion which are always used in the sense of net movement. The concept of diffusion as a net movement is also in harmony with the accepted meaning of the term diffusion coefficient, discussed later.

Diffusion pressure gradient

The meaning of this term should be clear from the following simple example. Consider a tube of uniform bore about a centimeter in diameter and several centimeters in length, at one end of which (A) a certain partial pressure of a gas is maintained, and at the other end of which (B) a lower partial pressure of that same gas is maintained. Regardless of the partial pressure distribution of any other kinds of gases present, diffusion of the gas will occur from A towards B, along a gradient of its partial pressures between the two ends of the tube, the pressure decreasing linearly between A and B. This gradient of partial pressures is also called a *diffusion pressure gradient*. The greater the difference in partial pressures between A and B for a given tube length, the "steeper" the gradient. Furthermore, the shorter the distance between A and B for a given difference in partial

pressures, the "steeper" the gradient. Other conditions remaining constant, the steeper the gradient, the faster the rate of diffusion. Steepness can be expressed quantitatively as the decrease in pressure per unit distance, for example, as diminution in partial pressure in atmospheres[2] per centimeter. The gradient just described is an example of a strictly linear gradient, i.e., one in which the diminution in partial pressure per unit length is constant for the entire gradient. Most diffusion pressure gradients, however, are non-linear. For example, if the tube through which the gas was diffusing had a larger diameter at one end than at the other, the gradient would be steeper toward the end of smaller bore. The diffusion pressure gradients of carbon dioxide, water-vapor, and oxygen between the stomates and the atmosphere are non-linear.

Diffusion pressure gradients are established and maintained during the diffusion of solutes, liquids, or solids as well as during the diffusion of gases.

Other terms for diffusion pressure gradient

Concentration gradient is often employed in essentially the same sense as diffusion pressure gradient. Disadvantages in the use of this term will be considered later in the discussion.

Diffusion coefficient

This term, or its synonym *diffusivity,* is the accepted expression for indicating the rate of diffusion of a substance. For a linear gradient, which will be the only case considered, it may be defined in general terms for a gas as the volume (or mass) of gas which diffuses across a unit area per unit of time per unit decrease of pressure per unit length. For example, BROWN and ESCOMBE (**5**) measured the diffusivity of carbon dioxide gas and expressed it as milliters of gas diffusing across 1 sq. cm. per sec. with a fall in the pressure of the gas of 1 atm. per cm. They found a mean value of 0.157 for the diffusion coefficient of carbon dioxide in the temperature range 7.7–13.3° C. and used such coefficients in computing the theoretical carbon dioxide "absorbing capacity" of a sunflower leaf.

Diffusion pressure

As previously mentioned the partial pressure of a gas is sometimes referred to as its diffusion pressure. Liquids can also be considered to possess a diffusion pressure (**20**) but the existence of such a physical property in a liquid becomes evident only under certain conditions as, for

[2] It is conventional to use the atmosphere as the unit for quantitative expression of partial pressures, diffusion pressures, osmotic pressures and related physical quantities, and this practice is followed in the present paper. Small pressures are often given as centimeters or millimeters of mercury. In the CGS system, however, the unit of pressure is the *bar* which originally was defined as a pressure of one dyne per square centimeter. Meteorologists have redefined the bar to designate a pressure of 1,000,000 dynes per square centimeter; a fractional unit being the *millibar* which equals 0.001 bar. One atmosphere equals 1.013 bars.

example, when a solvent and a solution are separated by a differentially permeable membrane. Solutes may also be considered to possess a diffusion pressure. Diffusion pressure is the cause of diffusion, not its result. Just as gases have a pressure whether or not any diffusion is occurring, so also do liquids and solutes have a diffusion pressure whether or not any diffusion is occurring. Diffusion pressure is therefore that physical property of a substance which is responsible for its diffusion whenever other prevailing conditions permit the occurrence of this process. Diffusion of a substance occurs only if a difference in its diffusion pressures exists between two contiguous regions under such circumstances that molecules can move from one region to the other. In any system diffusion always takes place from the region or regions of greater diffusion pressure of the diffusing substance to the region or regions of its lesser diffusion pressure.

Discussion of the diffusion pressure of liquids will be restricted to water and aqueous solutions, although the principles considered also apply to other liquids. If pressure is imposed on water, as by a piston in a closed system, or by the confining effect of the walls of a closed osmometer or of a plant cell when the enclosed volume of water is expanding as a result of osmosis, the diffusion pressure of the confined water increases by the amount of the imposed pressure. If the imposed pressure is 15 atm., for example, the diffusion pressure of the water increases by 15 atm. Correspondingly, if water is subjected to a "negative pressure" (tension) its diffusion pressure decreases by the amount of the negative pressure. The effect of the imposed pressure on the diffusion pressure of the liquid is quantitatively the same whether the liquid is pure, or whether solutes are present. In a solution the diffusion pressure of any solute present is also changed by the amount of the imposed pressure.

If water is confined under such conditions that its volume cannot increase and its temperature is then raised, this is equivalent to putting it under pressure, and the diffusion pressure of the water increases. If free expansion of the water is possible, i.e., if externally imposed pressure is kept constant instead of volume, increase in temperature has relatively little effect on its diffusion pressure. It is noteworthy that a change in temperature of water at constant pressure sufficient to markedly increase or decrease its vapor pressure usually has only a slight effect on its diffusion pressure. Effects of temperature upon the diffusion pressure of gases and water are discussed by CURTIS (**10**). There is also some pertinent discussion in a paper by URSPRUNG (**40**).

In an aqueous solution the magnitude of the diffusion pressure of the water is influenced by a third factor, the proportion of solute particles to solvent molecules. The diffusion pressure of water is diminished by the presence of solute molecules or ions and, within a wide range of concentrations, due account being taken of the hydration of solute particles, this diminution in diffusion pressure is closely proportional to the ratio of solute particles to solvent molecules. The diffusion pressure of the molecules or ions of any solute is also in part a function of this ratio.

Interpretation of all diffusion phenomena on the basis of differences in diffusion pressures would seem to be a logical and desirable procedure. This term can, in fact, be used to good advantage in the analysis of diffusion phenomena in gases. In liquids and solutes, however, it is difficult to make exact measurements of the magnitudes of diffusion pressures. This makes it unfeasible to use this physical quantity as such in the quantitative interpretation of diffusion phenomena involving liquids and solutes.

It is often possible, however, to measure quantitatively the amount by which the diffusion pressure of the water in a given solution is less than that of pure water at the same temperature and under atmospheric pressure. This quantity, sometimes called the "diffusion pressure deficit" (see later), can be used in the interpretation of osmosis and other diffusion phenomena in which water molecules participate even if we do not know the exact magnitude of the diffusion pressure of the water. In this indirect fashion, therefore, it is possible to use the concept of diffusion pressure in the analysis of diffusion phenomena in water and other liquids.

Other terms or concepts for diffusion pressure

Partial Pressure

Among physical scientists partial pressure is the standard term, when referring to gases, for the physical quantity which has been referred to in the immediately preceding discussion as diffusion pressure. This term has sometimes been applied to liquids with an analogous meaning but this usage is not very appropriate and should be discouraged.

Osmotic Pressure

This term has been used by some writers in a sense equivalent to that of diffusion pressure. Later discussion in this paper will show that this usage is not to be recommended.

Concentration

Diffusion phenomena are often interpreted on the basis of differences in *concentration* or in terms of *concentration gradients* of the diffusing substance. The term is used with different connotations by different writers. Some use concentration in the sense of number of molecules per unit volume; others in the sense of molar or molal concentration. In certain systems diffusion of a substance may be correctly described as occurring from regions of its greater to regions of lesser concentration, when concentration is defined as number of molecules per unit volume. Such explanations are frequently not valid, however, as many examples can be cited both in isothermal and in non-isothermal systems, in which the diffusion pressure gradient is opposite in direction to the concentration gradient. For critical interpretations, therefore, this term is not to be recommended, although its use may be permissible in simplified approaches to the concept of diffusion in elementary courses.

Attempts to analyze diffusion on the basis of molar or molal concentrations can only lead to ambiguities or misinterpretations, because there is no simple or constant relationship between the diffusion pressure of a solute or a solvent and the standard expressions for the molarity or molality of a solution. This statement does not apply, however, to the term "equivalent molality" (see later).

Specific free energy

Edlefsen (**14**) has emphasized the suitability of this concept in the interpretations of soil moisture problems. The specific free energy of the water at any point in a given system is considered to be the algebraic sum resulting from (1) the hydrostatic pressure it is under, (2) the presence of dissolved substances, (3) the operation of a force-field such as a gravitational or adsorptive field and (4) the effect of a surface or interfacial tension. Edlefsen further suggests, in the interest of an integrated picture of soil and plant water relations, that it might prove profitable to extend this concept to the analysis of soil water-plant relations, to the problems of movement of water through the plant, and to transpiration problems.

The quantity, "specific free energy," is neither conceptually nor dimensionally the same as diffusion pressure. In the CGS system specific free energy has the physical dimensions of ergs per gram, while diffusion pressure has the dimensions of dynes per square centimeter. The two units are thus incommensurable.

While the quantity specific free energy is probably well suited for certain thermodynamic interpretations of soil moisture relations, the desirability of the extension of this concept to plant water relations is dubious. The plant physiologist is often confronted not only with the problem of evaluating the direction of movement of water in plants but also, as in certain phases of the growth process, in the movement of plant organs, and in the behavior of guard cells, with the magnitude of the turgor pressures developing in plant cells. Often both the direction of movement of water and the magnitude of the turgor pressures prevailing in certain cells must be evaluated in order to obtain insight into the dynamics of a given physiological situation. Hence pressure units are better suited to the vocabulary of the plant physiologist than energy units. With a system of terminology which employs pressure units both water movement and turgor phenomena as they occur in plants can be described and interpreted.

It would seem just as useful, and perhaps more so, to extend the diffusion pressure concept (which is discussed in more detail later) from the plant into the soil, as has actually been done by some workers, than to extend the terminology of a specific free energy from the soil into the plant.

Activity

This term is used by some writers with a significance which is roughly analogous to diffusion pressure or to specific free energy. Diffusion of any substance such as water is considered to occur from regions of its greater to

regions of its lesser activity. This term can be used without ambiguity for qualitative designations of the thermodynamic state of water of other substances, but is not very suitable for quantitative interpretations of diffusion phenomena as they occur in plants.

Quantitatively expressed, the *activity* (or *activity coefficient*) of a substance is the ratio of its fugacity in a given state to its fugacity in some standard state which is usually given a value of unity (**26**). ***Fugacity*** is defined as the ideal or corrected vapor pressure of a substance (i.e., its vapor pressure if its vapor behaved as an ideal vapor) and is a measure of its "escaping tendency." Fugacities can be calculated in other ways than from vapor pressures and the concepts of fugacity and activity coefficient are well-known and widely used in thermodynamical evaluations, not only of water, but of many other substances.

In water at ordinary temperatures the vapor pressure is essentially identical in value with its fugacity. Hence the activity coefficient of the water in a solution is equal to the ratio of the vapor pressure of the water in the solution to the vapor pressure of pure water at the same temperature, pure water under atmospheric pressure being taken as the standard condition. For example, in a solution with an osmotic pressure of 100 atm. at 0° C. the activity of the water is 0.919. For a given solution the activity coefficient is virtually constant over the range of temperatures within which water is a liquid.

There are several disadvantages to the use of activity coefficients for giving quantitative expression to the dynamics of diffusion phenomena involving water: (1) In systems which are not isothermal use of this unit in the analysis of certain diffusion phenomena can easily lead to misinterpretations. (2) The "working range" of the activity scale commonly encountered in plants is only a very small part of the entire scale. A difference of 10 atm. in osmotic pressure would usually represent a very steep gradient in a plant yet the difference in activity coefficient between zero and 10 atm. osmotic pressure is only 0.008 at 30° C. Some examples of steep activity gradients can be cited for plants, however. Such a dynamic situation develops, for example, when dry seeds with water-permeable seed coats come in contact with water. (3) While the direction of movement of water can be interpreted on the basis of differences in activity coefficients, the dimensions of this term are not such as to permit the evaluation of turgor phenomena in plant cells. The importance of being able to do this has already been stressed.

Osmosis

If an aqueous solution is separated from pure water by a membrane permeable only to water, diffusion of water will occur across the membrane towards the solution because of the greater diffusion pressure of the pure water than of the water in the solution. This is an example of *osmosis* which may be conveniently, but not rigidly, defined as the diffusion of a solvent across a differentially permeable membrane. Osmosis may be regarded as

a special case of diffusion. In general, osmosis occurs whenever two solutions with a common solvent, in which the diffusion pressures of the solvent are different, are separated by a membrane more permeable to the solvent than to the solutes. Diffusion of the molecules or ions of a solute through a membrane is also a common phenomenon, but occurs independently of the diffusion of the solvent molecules, in accordance with differences in the diffusion pressure of the solute, and seldom if ever in amounts exactly proportional to the diffusion of the solvent.

The movement of water out of a plant cell when immersed in a solution with a higher osmotic pressure than the diffusion pressure deficit of the cell sap (see later), resulting in loss of turgor or in extreme cases in plasmolysis, and the movement of water into a flaccid or plasmolyzed cell when immersed in pure water or a hypotonic solution are well known examples of osmosis. The movement of water from the soil into the epidermal cells and root hairs of roots and the passage of water from cell to cell in the living tissues of a plant have generally been considered to be largely, and certainly are at least partly, osmotic phenomena.

It is generally assumed or implied in discussions of osmosis that the systems considered are isothermal. However, such temperature gradients as exist in plant tissues appear, in the light of available evidence, to have only a slight effect upon the osmotic movement of water in plants (**10**) and hence probably can be disregarded.

The term osmosis is also sometimes applied to the diffusion of a gas or of a solute across a membrane. Such usage, if widely employed, would generalize the term to refer to any diffusion process which takes place through a membrane, and makes the presence of the membrane the criterion of the process rather than the physical condition of the diffusing substance. Historical considerations, however, give priority to the employment of osmosis for the diffusion of a solvent across a membrane. Furthermore, certain aspects of this phenomenon appear to be so distinctive that it is convenient to have a distinguishing term for it. Restriction of osmosis to this meaning, however, is a purely arbitrary limitation, as fundamentally the diffusion of the molecules of gases and solutes through a membrane is analogous with the diffusion of the molecules of a solvent through a membrane.

Osmotic pressure

Probably more different meanings have been attached to this term than to any other in the lexicon of diffusion terminology (**17**). Historically—as the sense in which the term has usually been employed by most of the better known investigators of the phenomenon (**3, 6, 18, 22, 29, 30**)—osmotic pressure has been used to designate the maximum pressure which develops within a solution under certain ideal conditions. The necessary "ideal conditions" are that the solution must be confined within a membrane permeable only to the solvent, that the membrane be immersed in the pure solvent (usually water), and that the construction of the osmometer and attached

pressure measuring apparatus be such as to permit the development of a pressure equilibrium without any appreciable dilution of the solution. This requires, among other conditions, an inelastic membrane. Since osmotic pressure varies with temperature, the temperature at which the measurement is made should also be stated.

Pressure develops within the solution in an osmometer only after osmotic movement of the solvent has occurred from the pure solvent in which the membrane is immersed into the solution enclosed within the membrane. According to this concept only when the pressure has attained its maximum (equilibrium) value is it designated as the osmotic pressure.

This concept of osmotic pressure is not a very useful one, referring as it does to a pressure which develops in a solution only under a very special and seldom attained set of circumstances. Furthermore, as discussed later, the term "turgor pressure" is adequate for characterizing all actual pressures—maximum or otherwise—which develop in osmotic systems. The term "osmotic pressure" can be more usefully employed as an index of certain physical properties of a solution. In part such usage is a derivative one from the usage described in the immediately preceding paragraphs, which holds historical priority. For example, an aqueous solution of sucrose standing in a beaker may be described as having an osmotic pressure of 20 atm. at 25° C. This index characterizes the solution in two ways.

In the first place, osmotic pressure is an evaluation of the *potential* maximum turgor pressure which will develop in that solution if it is permitted to come to equilibrium with pure water in an ideal osmotic system at this temperature. In other words, if the solution *were* to be enclosed in an osmometer under the ideal conditions specified above, at equilibrium, a pressure of 20 atm. would prevail in the solution.

In the second place the osmotic pressure is an index indicating quantitatively the amount by which the diffusion pressure of the water in the solution is less than that of pure water at the same temperature and under atmospheric pressure.[3] Osmosis occurs because of the greater diffusion pressure of the solvent molecules on one side of the membrane than on the other and continues until the two diffusion pressures become equal. When the maximum turgor pressure has been attained in a solution enclosed in an osmometer under the previously specified "ideal" conditions an equilibrium has been established between the diffusion pressure of the water in the solution and the diffusion pressure of the pure water in which the osmometer is immersed. The osmotic pressure of a solution is, therefore, an index of the turgor pressure which must prevail in that solution if the diffusion pressure of the water in the solution is to be raised to a value equal to that of pure water at the same temperature under atmospheric pressure. Mathematically, therefore, the osmotic pressure is equal to the initial (i.e., before any osmosis

[3] The diffusion pressure of pure water at atmospheric pressure and at the same temperature as the water, the diffusion pressure of which is to be designated, is conveniently taken as the zero point on the diffusion pressure scale.

actually occurs) excess of the diffusion pressure of the pure water over the diffusion pressure of the water in the solution, assuming both to be at the same temperature. In other words the term is an index of the reduction in the diffusion pressure of the water in a solution as compared with the diffusion pressure of pure water, insofar as this results from the presence of solutes. Osmotic pressure, therefore, is an expression of the magnitude of the diffusion pressure deficit (see later) of the water in a solution resulting from the presence of solutes.

At maximum turgor pressure, therefore: OP = TP; at zero turgor pressure: OP = DPD. Furthermore, as emphasized by Crafts (**8**) at any intermediate turgor pressure: OP = TP + DPD. This is a transposition from another more familiar form of this equation which will be considered later.

Frequent use is made of the index concept of osmotic pressure in the analysis of physiological problems. Occasions are frequent when it is necessary to refer to the osmotic pressure of the cell sap. In cells with elastic walls this is not a constant quantity, even when no changes occur in the total number of solute particles in the cell sap, because of shifts in the volume of water present. This fact has led to the use of such phrases as "osmotic pressure at incipient plasmolysis" and "osmotic pressure at cell saturation," which are necessary in certain detailed analyses of the osmotic relations of cells. It is also frequently necessary to take into consideration the osmotic pressure of environmental solutions, such as the soil solution, fresh or ocean water, culture solutions, or plasmolytes in evaluating the physiological behavior of plants.

As is well known, the osmotic pressure of a solution varies with temperature, although not markedly. Many physico-chemical tables or calculations are based on osmotic pressures at 0° C. For plant physiological purposes it is preferable to give osmotic pressures as for a temperature of 20 or 25° C. If osmotic pressures are given for 25° C. the maximum by which they can be in error within the temperature range 15–35° C. is 3.4 per cent.

The term "osmotic pressure" has not been used by all writers with the meaning recommended in the preceding discussion. Osmotic pressure is sometimes used to designate the actual pressures which develop in plant cells or osmometers as a result of osmosis. If this usage were to be accepted a given solution could not be considered to possess an unique osmotic pressure at a given temperature but only a range of possible osmotic pressures. A solution which, in the meaning given preference in the foregoing discussion, has an osmotic pressure of 15 atm. might, in this sense of actual pressure developed, possess any possible osmotic pressure between zero and 15 atm., depending upon physical circumstances. As previously mentioned, the preferred term for actual pressures developing as a result of osmosis is "turgor pressure" (see later).

Osmotic pressure is sometimes defined as the mechanical pressure which must be imposed on a solution in order to prevent it from increasing in volume when placed under the ideal conditions specified previously [see for

example, HALL (**21**)]. Such an imposed pressure must be mathematically equal to the pressure which develops within the solution. Such a statement amounts to an indirect definition of osmotic pressure as the maximum pressure which develops in a solution under ideal conditions, which, as has been previously indicated, is not the most useful one.

EYSTER (**16**) and some others have advocated the use of osmotic pressure with a meaning essentially equivalent to the term "diffusion pressure" as already discussed. This confusion between the two concepts of diffusion pressure and osmotic pressure is not uncommon. Any such usage should, however, be discouraged since there does not seem to be any sound historical, theoretical, or experimental basis for the adoption of this point of view.

BROOKS (**4**) has advocated the use of the term "osmotic pressure" in a sense essentially equivalent to that of "diffusion pressure deficit" (see later). Although this suggestion logically appears to have some merit, actually there are very serious objections to its adoption as will be pointed out in the subsequent discussion.

Other terms or concepts for osmotic pressure

Some writers object to the use of osmotic pressure in the index sense advocated in the preceding discussion on the grounds that it does not represent a "real" pressure and a number of substitute terms such as "osmotic potential," "osmotic power," "osmotic concentration," and "osmotic value" have been suggested. In the mathematical sense, however, osmotic pressure must be given the dimensions of a pressure, as is clearly shown by the equations discussed at several points in this paper. The first three of the terms listed above have incorrect physical dimensions, if expressed in atmospheres or other pressure units, and the fourth one is physically undefined. Furthermore, at least two of these terms are used by some plant physiologists in other senses.

URSPRUNG (**39**) employs the term "osmotic value" as the designation for the "molal expression of the concentration of the plasmolyte which is isotonic with cell sap." From his discussion, however, it would appear that he actually means molar instead of molal.

CURTIS (**11**) advocates use of the term "osmotic concentration," expressed in terms of "equivalent molalities." The *equivalent molality* of a solution is defined as the molality of the ideal solution with which it would be in equilibrium. Osmotic concentration and osmotic pressure are not synonymous terms since their physical dimensions are different. Nevertheless, osmotic concentration, in this sense, is a valid concept and can be acceptably used as a substitute for osmotic pressure in the interpretation of osmotic phenomena. Unlike its osmotic pressure the equivalent molality of a solution does not vary with temperature; for this reason the later term can be employed to advantage in certain types of evaluations. If this term is used, however, transpositions to pressure units are necessary in the interpretation of many diffusion phenomena. It would seem simpler to select

terms for the fundamental osmotic quantities such that they all have the physical dimensions of a pressure.

Turgor pressure

In an osmotic system such as a vacuolate plant cell or closed osmometer pressures equal in magnitude to the osmotic pressure of the cell sap or enclosed solution seldom develop. The principal reason for this is that the ideal conditions under which the maximum pressure can develop do not often obtain. The long established name for the *actual* pressures which prevail within most plant cells is *turgor pressure* and there seems to be no substantial reason why this term cannot also be applied to the similar pressures developing within purely physical systems. As will be made clear in the later discussion, it is sometimes possible for turgor pressures to have a negative value.

Wide use is made of the concept of turgor pressure in the analysis of problems of stomatal behavior, wilting phenomena, growth (particularly cell enlargement), and of plant movements such as phototropism and hydrotropism. When the osmotic pressure and diffusion pressure deficit (see later) can be measured accurately it is possible to calculate the actual magnitude of the turgor pressure of plant cells and to use such values in the quantitative evaluation of physiological phenomena.

Other terms for turgor pressure

As previously noted, the term "osmotic pressure" is sometimes employed with a meaning equivalent to that of turgor pressure as the latter is used in this discussion. If "osmotic pressure" is used in the recommended index sense the two terms refer to entirely different physical quantities which should be kept distinct. Two plant cells in which the cell sap has the same osmotic pressure may possess entirely different turgor pressures and correspondingly two plant cells in which the cell sap has different osmotic pressures may possess the same turgor pressure.

Wall pressure

In the usual type of mature plant cell turgor pressure is exerted against the protoplasmic layer and in turn transmitted to the cell wall. The wall is thus subjected to a pressure equal in magnitude to the turgor pressure. In cells with elastic walls this often results in outward distention of the wall. The wall can be considered as exerting a compressional effect on the cell contents, often referred to as *wall pressure*. When turgor pressure is negative (i.e., the cell sap is under tension) the wall pressure is likewise negative, the wall being subjected to a centripetal pull because of its adhesion to the protoplasmic layer and cell sap mass within the cell. Some analysts of the dynamics of cell water relations make extensive use of the concept of wall pressure. Because of the invariable equality, however, both in sign and magnitude, of wall pressure and turgor pressure, it would seem that this term could be dispensed with in the interpretation of cell water relations.

Diffusion pressure deficit

Although osmotic pressure is distinguished above all other diffusion terms for the number of different concepts which have become associated with a single phrase, the quantity designated in this paper as the "diffusion pressure deficit" [or "pressure deficit" (see later)] is distinguished above all others for the number of different terms which have been proposed for what is essentially a single concept.

The diffusion pressure deficit of water, as pointed out previously, is the amount by which its diffusion pressure is less than that of pure water at the same temperature and under atmospheric pressure. In isothermal systems the diffusion pressure and hence diffusion pressure deficit of water are influenced only by the concentration of solutes present and by the pressure prevailing in the water or solution. The osmotic pressure of a solution is the index of its diffusion pressure deficit insofar as this results from the presence of solutes. Imposition of a positive pressure on water or a solution increases the diffusion pressure of the water and hence decreases its diffusion pressure deficit by the amount of the imposed pressure; subjection of the liquid to a "negative pressure" (tension) correspondingly decreases the diffusion pressure of the water and hence increases its diffusion pressure deficit.

For a vacuolate plant cell the fundamental relations among the important osmotic quantities are expressed by the equation:

$$DPD = OP - TP \quad (WP = TP)$$

In a cell with a sap OP of 12 atm. and a TP of 5 atm. the DPD would be 7 atm. In a flaccid cell with a zero TP: DPD = OP. In a completely turgid cell: TP = OP; hence DPD = 0.

As previously mentioned, in at least some kinds of cells, and even more commonly in xylem ducts, water may pass into a state of tension (**7, 13, 15, 31**). The WP and TP of such cells or vessels both have a negative value equal to the tension; hence in such cells DPD = OP – (–TP).

When, as is often the situation in plant tissues, a given cell is subjected to compression by surrounding cells, the pressure thus exerted against the cell increases the turgor pressure of the cell sap. Such a cell thus acquires a lower diffusion pressure deficit than it would possess were it isolated from the tissue in which it is located.

Objections are sometimes raised against the use of osmotic pressure as a term in this equation on the grounds that the diffusion pressure deficit in plant cells is partly of imbibitional origin and in some kinds of cells may be entirely so. URSPRUNG (**39**) distinguishes between "suction force of the cell" (equivalent to diffusion pressure deficit) and "suction force of the cell contents." This latter term is used by him in an equation analogous to that given above, apparently at least partly in recognition of the rôle of imbibition as a component in determining the diffusion pressure deficit of cells. However, in all cells with relatively large vacuoles, osmotic effects are determinative; the diffusion pressure deficit of the protoplasm or other imbibants tends to equilibration with the osmotic pressure of the cell sap. Even

in cells in which the diffusion pressure deficit is entirely or predominantly of imbibitional origin its value can be stated in terms of the equivalent osmotic pressure so the use of this term in the equation would seem to be permissible for all kinds of cells.

In cells with elastic walls the osmotic pressure is not a fixed quantity even if the absolute number of solute particles in the cell sap remains constant. In general, the more turgid (the larger) a cell of this type, the less its osmotic pressure. Variations in the volume of such a cell in turn necessarily influence its diffusion pressure deficit, causing it to be greater in magnitude in the flaccid cell (cell at incipient plasmolysis) than it would be if no change occurred in the volume of the cell. Correspondingly the turgor pressure of such a cell when fully turgid is less than it would be were there no increase in its volume. The influence of changes in cell volume upon the osmotic quantities of plant cells have been fully analyzed by HÖFLER (**23**) BECK (**2**), and URSPRUNG (**39**). In some plant cells, however, the cell wall is virtually inelastic and in many others is nearly so. In simplified evaluations of cell water relations the effect of variations in cell volume, which is often negligible, is frequently disregarded.

The diffusion pressure deficit of plant cells is not a purely abstract quantity; it is one which, in many kinds of cells at least, is actually measurable, at least with fair approximation.

The fundamental significance of the diffusion pressure deficit concept becomes apparent whenever attempts are made to interpret movements of water into, out of, or within plants. When the diffusion pressure deficits of two adjacent cells are unequal (when a gradient of diffusion pressure deficits exists) water invariably diffuses from the cell of lesser to the one of greater diffusion pressure deficit, unless some interfering or offsetting mechanism prevents or counteracts such a movement.

The justification for employing the concept of diffusion pressure deficit in interpreting the diffusion of liquids rather than the more direct concept of diffusion pressure has been discussed previously.

Other terms and concepts for diffusion pressure deficit

SUCTION FORCE

Suction force (of the cell). This was the term used by URSPRUNG and BLUM (**41**), and seems to have historical priority among all the expressions which have been suggested for the quantity which in the previous discussion has been designated as the diffusion pressure deficit. There are, however, several objections to this term. The first and most serious is that its physical dimensions are incorrect. The physical dimensions of this quantity are such that it should be expressed in pressure units (force per unit area) and it should be named accordingly. The implications of the word "suction" are also undesirable; it is inconsistent with the accepted concept that the water molecules themselves are the active agents in any diffusion phenomenon in which they participate. This objection has been well presented by SHULL (**33**).

Water absorbing power

This term (**37**) does not have the correct physical dimensions and has not been widely used.

Suction pressure

This term (**36**) has the correct physical dimensions but there are grave objections to the word "suction" as previously pointed out. Furthermore, the phrase is inherently self-contradictory and unsatisfactory for that reason.

Suction tension

While this term (**2**) has the correct physical dimensions, it involves the unfortunate implications of the word "suction." Furthermore, among students especially, the occurrence of the word "tension" in this term is likely to be confusing. That actual tensions may develop in the sap in some kinds of plant cells and in xylem ducts is well known and has been referred to in the previous discussion. Employment of the word tension in both this sense and in the concept implicit in "suction tension," which may or may not designate conditions under which actual tensions exist, is likely to result in ambiguity.

Turgor deficit

This term was first used by Curtis and Scofield (**12**) and is defined by Curtis (**11**) as equal to the difference between the actual turgor in a cell and the maximum turgor which can prevail in that cell at the same volume. The only objection which can be raised against this term is that it is not very appropriate except for cells or osmometers, and it is desirable to have a term which is equally suitable for solutions, imbibants, cells and soils, in order that a consistent and unified interpretation of plant water relations may be possible.

Net osmotic pressure

This has been advocated by Shull (**35**) and Lyon (**27**) as a term for the physical quantity under discussion. This term cannot be applied very appropriately to imbibants or soils. Furthermore, use of the term "net osmotic pressure" sometimes involves one in the logically awkward situation of recognizing that in certain plant cells this quantity may exceed the osmotic pressure. This would be true, for example, in a cell with an osmotic pressure of 10 atm. in which the cell sap was under a tension of 5 atm.; such a cell would have a "net osmotic pressure" of 15 atm. Or, to cite a more extreme example, water in a xylem tracheid or vessel with an osmotic pressure of virtually zero, but under a tension of 10 atm., would necessarily be considered to have a net osmotic pressure of 10 atm. While this is not an entirely irrational procedure, it does represent a mental hazard to clear thinking on this problem, especially to students, and hence weakens the desirability of this term.

Effective osmotic pressure

An earlier suggestion by Shull (**34**) as a designation for this physical quantity, does not involve the mental paradoxes which would sometimes be encountered in the employment of "net osmotic pressure." Of these two terms, "effective osmotic pressure" would, therefore, seem to be the preferable one. Like "net osmotic pressure," however, this term cannot very appropriately be applied to imbibants or soils.

Osmotic pressure

Brooks (**4**) has advocated use of this term in a sense equivalent to that of diffusion pressure deficit. This is an apparent simplification which only contributes to confusion in thinking and cannot be recommended. A point which does not always seem to be appreciated is that it is necessary to employ three different physical quantities, each conceptually different, in order to characterize adequately the osmotic relations of plant cells. These three quantities are, using each term in the sense recommended in the previous discussion: (1), osmotic pressure; (2), turgor pressure; and (3), diffusion pressure deficit (or pressure deficit—see later). It is the osmotic pressure of the cell sap at incipient plasmolysis, for example, not its diffusion pressure deficit, which determines what the concentration of a plasmolyte must be before plasmolysis occurs. In analysis or interpretations of the direction of the movement of water in plants the diffusion pressure deficits of various cells, not their osmotic pressures, are the significant quantities. And finally, quantitative expressions of the differences in the turgor of plant cells, important in the evaluation of many of the physiological phenomena of plants as previously noted, can be made only in terms of turgor pressure. If this suggestion for the meaning of the term "osmotic pressure" should be accepted it would be necessary to adopt some other term for the quantity we now call by this name. The only possible outcome of following such a course would be the adoption of a different but just as complicated a terminology which would be less consistent with the historical development of thinking on this subject than the terms which are given more favorable consideration in this discussion.

Diffusion pressure deficit

The designation used for this physical quantity in the present discussion, (**28**) is a term which can logically be derived from an analysis of diffusion phenomena in terms of diffusion pressures. This term has the correct physical dimensions. It can be applied without qualification to cells, solutions, imbibants, and soils, thus aiding in the expression of a unified point of view in the analysis of all phases of the osmotic movement of water in relation to plants. Furthermore, the phrase implies that water molecules are the active agents and includes no words with undesirable connotations such as "suction." Perhaps the most serious objection to "diffusion pressure deficit" is that it is a somewhat unwieldy term. This objection can be met

by using a suitable abbreviation such as DPD or "pressure deficit" (see later).

Some have criticized "diffusion pressure deficit" on the grounds that it is a negative term, failing to realize that this is one of the advantages of the phrase rather than a disadvantage or objection. It emphasizes the fact that the diffusion pressure of the water in plants is usually less than that of pure water at the same temperature and under atmospheric pressure. By implication this indicates that the osmotic movement of water into and through plant tissues is at least largely a result of the "relative negativity" of the diffusion pressure of the water in plant cells. Occurrence of negative diffusion pressure deficits in plants is rare; the only common example is when the sap in xylem cells or vessels is subjected to a "root pressure."

Pressure deficit

This is another possible term for this physical quantity. Although "diffusion pressure deficit" has been used throughout this article, it would seem that "pressure deficit" is at least as good and perhaps a better term. Several of the reviewers of this paper have, independently of the author, suggested this as a suitable term. It can be regarded as simply an abbreviation for "diffusion pressure deficit" although perhaps not all of its advocates would favor such a derivation of the term. "Pressure deficit" possesses all the advantages of "diffusion pressure deficit" plus the added one of greater concision.

Imbibition

Upon immersion in water many relatively dry organic materials swell markedly, often to many times their original volume and weight. Examples of such substances are agar, starch, kelp stipe, gelatin, and seeds with water-permeable seed coats. The increase in volume results from a movement of water into the substance. This process is sometimes referred to simply as swelling but is more often designated as *imbibition*.

The mechanism of imbibition is undoubtedly very complex. It appears quite certain that capillary forces play an important rôle in the process. Specific intermolecular affinities or compatibilities are also involved as is shown by the fact that certain imbibants imbibe only certain liquids. Agar, for example, imbibes water but does not imbibe any of the common organic liquids. Rubber and certain plastics, on the contrary, do imbibe many organic liquids but do not imbibe water.

Nevertheless, imbibition exhibits certain dynamical features in common with diffusion phenomena, and the direction of diffuson of a solvent from a solution into an imbibant or vice-versa appears to follow essentially the same principles which hold for diffusion phenomena in general. Movement of water into an imbibant may be interpreted as occurring because of the greater diffusion pressure of the water in the liquid than in the imbibant. The less the diffusion pressure of the water in the external solution, the smaller the quantity of water which will be imbibed by a given imbibant be-

fore a dynamic equilibrium is attained. Much less water will be imbibed by a dry seed with water-permeable seed coats when immersed in a solution with an osmotic pressure of 100 atm., for example, than by a similar seed when immersed in pure water (**32**). When a dynamic equilibrium has been attained, the diffusion pressure deficit of the water in the imbibant is equal in magnitude to that of the water in the external fluid. If the osmotic pressure of a circumambient solution is sufficiently high there is no diffusion of water into the imbibant; i.e., no imbibition occurs. Most of the foregoing statements apply strictly only when the complication of diffusion of solute molecules into or out of the imbibant does not occur. When, as often happens, this takes place, interpretation of the dynamics of the system becomes more complex but the principles just described still hold, although their operation may sometimes be obscured.

Imbibition pressure

That pressures ranging up to at least 1000 atm. can develop during the process of imbibition has long been known. The existence of such pressures does not become apparent unless the imbibant is confined; they are thus analogous with the turgor pressures which develop in solutions which are enclosed in plant cells or osmometers. Diffusion of water into either a solution (osmosis) or an imbibant (imbibition) results in an increase in the volume of the solution or imbibant provided that the system is not rigidly confined. Such diffusion results in the development of pressure, usually accompanied by a limited increase in volume, if free expansion of the system is restricted.

The *actual* pressure which develops as a result of the swelling of an imbibant is sometimes called *imbibition pressure*. Such actual pressures only become measurable or apparent under certain special circumstances. It would seem preferable, however, to employ the term imbibition pressure with a meaning analogous to that of osmotic pressure; that is, as an index of certain properties of the imbibant rather than in the sense of an actual pressure. Used in this way, imbibition pressure becomes an index of (1) the potential maximum pressure which can develop in the imbibant as a result of imbibition; and (2) the diffusion pressure deficit of the water in the imbibant as long as the free expansion of the imbibant is not impeded in any way. Different degrees of hydration of the same quantity of imbibant result in different imbibition pressures just as different amounts of solvent associated with the same quantity of solute result in different osmotic pressures. In either an imbibitional or an osmotic system the greater the amount of water present in proportion to a given amount of imbibant or solute, the less the imbibition or osmotic pressure. Half-swollen pea seeds, for example, have a smaller imbibition pressure, in the index sense, than air-dry pea seeds; pea seeds which have reached the condition of maximum swelling have an imbibition pressure of zero.

There seems to be no clearly established term for the actual pressures

exerted by a swelling imbibant against the walls of any confining system, but there appears to be no reason why they, as well as the actual pressures which develop as a result of osmosis, should not be termed turgor pressures. The turgor pressures developed in an imbibant seldom become equal to the imbibition pressure of the imbibant.

Vapor pressure

The pressure exerted by a vapor, analogous to the gas pressure of gases, is called vapor pressure. Plant physiologists and ecologists seldom have occasion to use this term except in reference to water vapor, hence this discussion will be confined to a consideration of aqueous vapor pressures.

A distinction must be made, however, between two different senses in which this term is used. Physicists and chemists usually employ it to refer to *equilibrium* or *saturation* vapor pressures; i.e., to the vapor pressure prevailing in a space after a dynamic equilibrium has been established between the water vapor in that space and liquid water. If an open dish of water is kept under a bell jar for some time under thermally stable conditions the vapor pressure in the enclosed atmosphere will attain its equilibrium value. At a given temperature the equilibrium vapor pressure of water has a certain value which is independent of the absolute amounts of vapor or water in the system. The saturation vapor pressure of water increases with increase in temperature, but the relation is not a linear one.

Meteorologists use "vapor pressure" as a term for describing moisture conditions in the atmosphere in the sense of the *actual* vapor pressure,[4] which is seldom equal to the saturation vapor pressure for the prevailing temperature. On a clear summer day in the east central United States, for example, the actual vapor pressure of the atmosphere is often in the vicinity of 15 mm. of mercury. Saturation vapor pressures corresponding to the commonly prevailing daylight temperatures on such days would lie within the range of 25–45 mm. of mercury. Use of "vapor pressure" with this connotation is more common among plant physiologists and ecologists than its employment as a designation for saturation vapor pressures. The context in which the term is being used should always be made clear in any discussion of vapor pressures.

Since the (actual) vapor pressure of water vapor is its diffusion pressure, this term is commonly employed in interpreting the movement of water along vapor pressure gradients from, for example, one part of a plant to another through the intercellular spaces, or from the intercellular spaces through the stomates into the atmosphere (**9, 31**). The concept of vapor pressure is also used in the analysis of the molecular dynamics of the phenomena of evaporation and condensation, two physical processes which are often of concern to the plant physiologist and ecologist in analyzing the physiological

[4] Vapor pressures are usually expressed in terms of their equivalent pressure in millimeters or inches of mercury. A previous footnote on the *bar* (1,000,000 dynes per square centimeter) as a unit of pressure in the CGS system should be recalled. One millimeter of mercury equals 1.33 millibars (0.00133 bar).

reactions or ecological distribution of plants. In general, vapor pressure is the most satisfactory unit in which to express humidity values in the analysis of physiological problems.

A commonly used synonym for vapor pressure is *vapor tension*. Since the word "tension" is often used with another connotation this term is less desirable than "vapor pressure."

Vapor pressure deficit

This term has been used by a number of writers in discussions of the diffusion and condensation of water vapor, and evaporation of water in relation to the physiological and ecological behavior of plants. It is defined as the difference between the actual vapor pressure of an atmosphere and its saturation vapor pressure at the same temperature. This concept is a valid one only for isothermal or near-isothermal system. Attempts to apply it in thermally heterogenous systems lead to misinterpretations; hence indiscriminate use of this expression must be avoided. For further evaluation of the utility and limitations of this concept, recent papers by ANDERSON (**1**), LEIGHLY (**25**), THORNTHWAITE (**38**), GORDON (**19**), and HUFFAKER (**24**) should be consulted.

Relative humidity

This, the most familiar of all terms used for characterizing the water vapor conditions in an atmosphere, is defined as the percentage saturation of an atmosphere. Since, at a given temperature, the vapor pressure of an atmosphere is proportional to the concentration of water vapor molecules present, the relative humidity may also be expressed as the ratio of the actual vapor pressure of the atmosphere to its saturation vapor pressure at the same temperature.

The relative humidity depends upon the two factors of concentration of water vapor in the atmosphere and temperature. The same relative humidity (60 per cent., for example) will therefore correspond to very different vapor pressures at different temperatures. At 20° C. the vapor pressure corresponding to this relative humidity would be 10.52 mm. Hg; at 40° C., 33.19 mm. Hg. Only in isothermal systems are relative humidities true indices of relative vapor pressures. Hence undiscriminating employment of relative humidity values in the interpretation of diffusion phenomena often gives rise to serious misconceptions. Examples of some of the misinterpretations which result from uncritical use of this term are given by ANDERSON (**1**) and CURTIS (**9**).

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NOTES

Professor C. A. Shull.—It may be of interest to members of the society to know that Professor C. A. SHULL has established his permanent residence in North Carolina. His address is 42 Oakwood Avenue, Asheville, North Carolina.

Life Members and Patrons.—Attention of members of the A. S. P. P. is called to the fact that the society offers life-membership on the payment of One Hundred Dollars in a lump sum. Life members pay no further dues and receive Plant Physiology for life without any additional charge. Members can become Patrons of the society upon payment of Two Hundred Dollars (or more at their option) and also receive the journal for life without further cost. There may be a distinct financial advantage in becoming a life member or patron of the society in view of present income tax rates and regulations. It is hoped that members may avail themselves of the opportunity to become life members or patrons.

Thomas Jefferson and the Scientific Trends of His Time.—This resumé by CHARLES A. BROWNE of the U. S. Department of Agriculture has been published as a recent number of Chronica Botanica. It is a sixty-page historical account of JEFFERSON's scientific activities, stressing especially his life-long interest in botany and support of its applied phases. The discussion of his political and scientific activities is well documented with interesting excerpts from his writings. His correspondence reveals that during the period of his public service as ambassador, secretary of state and president he continued to give the influence of his high official position to many notable scientific undertakings. A selected bibliography of sixty-eight titles on Jefferson's work in and on the behalf of science is included. The publication is available at $1.25 through the Chronica Botanica Company, Waltham, Mass.

Chemical Testing of Plant Nutrient Solutions.—This is a sixty-eight-page laboratory manual by C. S. FAWCETT and R. H. STOUGHTON. It gives complete quantitative colorimetric procedures for the determination of the various inorganic ions, including trace elements, in the ranges in which these are commonly found in plant culture solutions. The format permits rapid reference use both for amateurs or hobbyists as well as for laboratory technicians. A brief bibliography devoted to the methods described is included. The book is available at nominal cost through the Tintometer Ltd., Salisbury, England.

Advances in Protein Chemistry, Vol. 1, 1944.—A concise but well-rounded consideration of recent progress in various branches of protein chemistry is included in this first volume of a collection of papers edited by

M. L. ANSON and JOHN T. EDSALL. Eight sections, contributed by outstanding investigators in each field, cover information on the nature of proteinaceous complexes in plants and animals. Future volumes contemplated by the editors will continue the review of current advances in protein research. This published volume is concerned with biological systems as they occur in nature. The second volume, which editors expect to publish in 1945, will emphasize the progress in research on protein nutrition during the war. An opportunity is being accorded specialists to present in *Advances in Protein Chemistry* a more elaborate statement of their views than is usually possible in scientific journals.

The first section of volume I on the lipoproteins in certain plant and animal components is written by ERWIN CHARGAFF of Columbia University who reviews the classification, properties, and structural nature of well-defined lipoproteins present in cellular an extracellular substances. FRANCIS O. SCHMITT of the Massachusetts Institute of Technology is the author of a section on the structural protein components of animal cells and tissues. He outlines chemical and physical techniques which have improved our knowledge of the ultrastructure and chemical character of these proteins.

The important rôle of serological methods in the determination of the chemical nature of proteins is discussed by HENRY P. TREFFERS of Harvard. The chemical basis of specificity methods, their interpretations, properties of antibodies, and other immunochemical principles are well documented and supplemented with an extensive list of references. The biochemistry of complexes formed by proteins with alkaline earth ions is presented by DAVID M. GREENBERG of the University of California Medical School. BACON F. CHOW of the Squibb Institute contributes a section on purification and properties of certain protein hormones describing chemico-physical methods of purification and various biological aspects of homogeneous protein preparations. DONALD S. PAYNE and L. S. STUART of the War Food Administration give the history and development of the soybean proteins for use as a human food. Technological and chemical data of import to the nutritionist, the physiologist, and the biochemist are included along with a concise account of the economic rise of the soybean plant through a period of nearly 5,000 years to its present place as a major war crop.

JESSE P. GREENSTEIN of the National Cancer Institute reviews nucleoproteins, their reactions, distribution, and cytochemical data. A discussion of muscle proteins by KENNETH BAILEY of the University of Cambridge comprises the final section. Extraction and quantitative techniques are reviewed. Properties and characteristics of muscle proteins, and their relation to muscle contractility are given with particular attention to myosin. The editors have succeeded in bringing together under one cover significant developments in various fields of protein chemistry and have compiled an extensive bibliography of great value to research workers in many fields of biology. The book comprises 341 pages and is available through the Academic Press, Inc., 125 East 23rd Street, New York, N. Y., at $5.50.

Chemistry and Industry of Starch, Starch Products and Related Compounds, 1944.—RALPH W. KERR of the Corn Products Refining Company in collaboration with fourteen co-authors has published a monograph containing the most recent physico-chemical information on starch, important starch products, and their technology. The volume is of interest to industrialists, technologists, plant scientists, and applied chemists. Twenty-two chapters are divided into five general sections, each reflecting a different approach to the subject of starch. These are: I, occurrence in nature; II, preparations; III, properties; IV, reactions; and V, uses.

The complete description of individual starch granules with accompanying photomicrographs is a valuable aid to the identification of common starches. A step by step procedure for the manufacture of corn, tapioca, potato, and other industrial starches is described. The physical and chemical properties of the heterogeneous physico-chemical components found in starches are outlined and followed by a consideration of the reactions of these constituents. An account of the condensation, oxidation, and hydrogenation of starch molecules and the resulting derivatives is presented. The numerous and varied uses of starches which are described indicate its indispensability in modern life. The final section deals with the most important uses, namely, in the manufacture of paper, textiles, adhesives, and in the fermentation and food industries. Each chapter concludes with a complete bibliography and patent list. These lists, with the cumulative authors' index, make this volume an extremely useful reference for scientists and industrialists interested in starches.

This text is an outstanding contribution in the field of carbohydrate chemistry. The subject matter is authentic and well arranged, the book is excellently illustrated, well indexed, and the bibliographies are comprehensive. The book contains a very useful resumé of the progress of carbohydrate research which paved the way for the starch industry. The volume comprises 471 pages. Copies are priced at $8.50 and may be ordered from the Academic Press, Inc., 125 East 23rd Street, New York, N. Y.

PREPARATION OF MANUSCRIPTS FOR PUBLICATION IN PLANT PHYSIOLOGY

The following suggestions are made to authors writing papers for this journal in order to minimize revisions and editorial corrections. Though many points may appear arbitrary or trivial, attention to details by authors is necessary to secure uniformity of format and style. The membership dues and subscription fees go almost entirely to publication costs, as all of our editors give their service without financial recompense. Hence the cooperation of authors in preparation of manuscripts conserves our funds and permits greater liberality as to length and number of papers published.

1. In the preparation of typescripts, authors are requested to examine a printed copy of the journal of Plant Physiology to note the position of headings, general organization, and the methods of citing literature particu-

larly, in order to bring the script into conformity with the general architecture of published papers.

2. Leave a 3-inch margin at the top of the first page, liberal side margins for editorial marks, and double spacing of lines so that there is space for any required editorial changes.

3. Avoid footnotes as far as possible. They are expensive. If you do use them, number them with Arabic numerals.

4. Place your name and figure number on the back of each figure. Do not put legends into or on the figure, either front or back. Type all legends, properly numbered to correspond to the figures, on a separate sheet of your manuscript. Graphs and drawings should be inked with fairly heavy lines to insure clarity of text figures after reduction.

5. Leave headings unscored, scientific names without underlining, etc. The editors will take care of all of these matters in accordance with the practice of the journal. Generic names used alone are not italicized. In general, do not underscore any lines.

6. Place each table on a separate page; do not crowd too much material into a single table. Note size of printed page (width and length, $4\frac{1}{2} \times 7$ in.) and gauge tables accordingly, leaving plenty of space in margins and in the interior for editorial marks. If at all possible, arrange tabular data horizontally on the page. Use horizontal lines at top and bottom and in column headings, but not in interior of tables. Use vertical lines between columns. Spaces (leads) are usually used in the final printed tables to separate sets of data. For footnotes to tables use the asterisk, dagger, double dagger, and section. Tabular material should be used sparingly. It costs over $10.00 per page. Author will be billed for the cost of tabular data in excess of 25 per cent. of the total paper.

7. Place acknowledgments at the end of the paper. The name of your institution and its address should also be given at the end of the manuscript.

8. Use separate pages for the literature cited. Give complete citations, author, title, journal name, volume number, inclusive pages, and year. (See any literature list in the journal for examples.) Punctuate according to the examples, and leave no citations incomplete. Write out names of states in full. Note proper abbreviations of journals.

An error frequently noted by our editors in manuscripts with long bibliographies is the failure to mention in the text all citations listed. Authors can assist in this matter by checking off the citations in the bibliography as they encounter reference to them in the body of the text in proofreading copy prior to submission.

9. Limit the size of all pages, drawings, tables, and photographs to standard $8\frac{1}{2} \times 11$ inches, or less.

10. Use mathematical formulae only when necessary, and be conservative in the use of space.

11. Authors will be billed for alterations in the galley proofs involving excessive changes from manuscript copy.

12. Send original copy of manuscript to editor, W. F. Loehwing, University of Iowa, Iowa City, Iowa. Retain a carbon copy to insure against loss, and to consult in case of need. We solicit your cooperation in these matters to relieve the editors and printers of some of their most frequent and costly difficulties.